# D D T

## Chemistry, Metabolism, and Toxicity

by
Ben H. Lim

Division of Enforcement Proceedings
Office of Enforcement and General Counsel
Environmental Protection Agency
Washington, D. C. 20460

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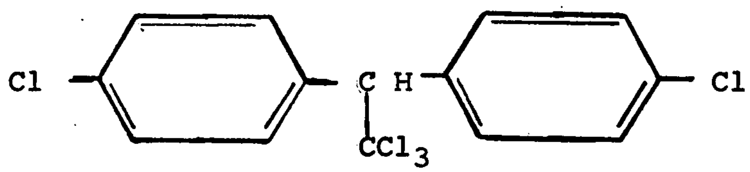
# DDT

DDT, Anofex, Arkotine, Chlorophenothane, Dedelo, Dichlorodiphenyltrichloroethane, Dicophane, DND, ENT 1506, Genitox, Gesapon, Gesarex, Gesarol, Guesarol, Gyron, Ioxdex, Kopsol, Neocid, Pentachlorin, p,p'-DDT, Rukseam, Zerdane, and $\alpha$, $\alpha$ - bis (p-Chlorophenyl)-B,B,B-trichlorethane are alternative names for a chlorinated hydrocarbon insecticide with the official chemical name of 1,1,1-Trichloro-2,2-bis (p-Chlorophenyl) ethane. The insecticide has a molecular weight of 354.50. Percentage wise, carbon is 47.43; hydrogen, 2.56; and chlorine, 50.01. The compound has the following chemical structure:



DDT consists of solid, white needle crystals with a melting point of 108.5-109°C and has a vapor pressure of $1.5 \times 10^{-7}$ mm of Hg at 20°C and $3.0 \times 10^{-7}$ mm of Hg at 25°C. Its solubility in water has been reported as low as 0.2 parts per billion (ppb), but the best estimate appears to be about 1.2 ppb or $3.4 \times 10^{-10}$ moles per 100 milliliters of water at 25°C. The insecticide is, however, soluble in many of the common organic solvents. Table 1 shows solubilities of DDT in some of the organic solvents at various temperatures.

Table 1

Solubility of DDT in some Organic Solvents at 27°C

| Solvent | Solubility (g/100g of solvent) |
|---|---|
| Acetone | 74 |
| Acetophenone | 65 |
| Benzene | 89 |
| Carbon tetrachloride | 28 |
| Chlorobenzene | 67 |
| Cumene | 43 |
| Cyclohexane | 19 |
| Cyclohexanone | 122 |
| Cymene | 34 |
| o-Dichlorobenzene | 45 |
| Dichloroethane | 47 |
| Dimethylphthalate | 29 |
| Dioxane | 89 |
| Dipentene | 26 |
| Ethyl alcohol (95% at 24°C) | 2.2 |
| Isoproryl alcohol | 14 |
| Methylene chloride | 66 |
| Methyl ethyl ketone | 100 |
| Methylnaphthalenes (mono- and di-) | 56 |
| Monomethylnaphthalene | 51 |
| Pinene | 16 |
| Tetrachloroethylene | 23 |
| Tetralin | 63 |
| 1,2,4-Trichlorobenzene | 28 |
| Trichloroethylene | 38 |
| m-Xylene | 64 |
| o-Xylene | 66 |

DDT was first described in 1874 by Othmar Zeidler in Germany. Its insecticidal effectiveness, however, was not discovered until 1939 by Paul Muller at the Basal laboratories of J. R. Geigy S.A. in Switzerland. It was patented in 1942. The insecticide was brought into the United States during the same year for testing. It was later imported in quantity. Because of the compound's insecticidal effectivesness, the United States began producing DDT in large quantities for military use by early 1944.

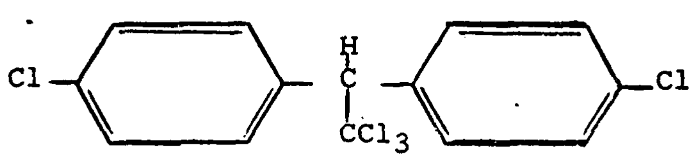
The technical grade product is a complex mixture of compounds in which the p, p' or the 4,4'-isomer amounts to 75-76%. In Table 2 the composition of three samples of technical grade DDT is given.

Table 2

Composition of Technical Grade DDT

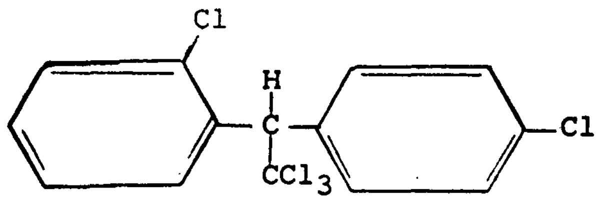
| Compound | Content (%) in samples with setting point 91.4°C | 91.2°C | 83.6°C |
|---|---|---|---|
| 1,1,1-Trichloro-2,2-bis (p-chlorophenyl)ethane 1/ | 72.7 | 72.9 | 70.5 |
| 1,1,1-Trichloro-2-(o-Chlorophenyl)-2-(p-chlorophenyl)ethane 2/ | 11.9 | 19.9 | 20.9 |
| 1,1,1-Trichloro-2,2-bis (o-Chlorophenyl)ethane 3/ | 0.011 | - | - |
| 1,1-Dichloro-2,2-bis (p-Chlorophenyl)ethane 4/ | | | |
| 1,1-Dichloro-2-(o-Chlorophenyl)-2-(p-Chlorophenyl)ethane 5/ | 0.17 | 0.3 | 4.0 |
| p-Chlorophenyltrichloromethylcarbinal 6/ | - | 0.2 | - |
| o-Chlorophenyltrichloromethylcarbinal p-chlorobenzene sulfonate 7/ | 0.57 | 0.4 | 0.1 |
| p, p'-Dichlorodiphenyl sulfone 8/ | 0.034 | 0.6 | 0.1 |
| o-Chlorophenylchloroacetamide 9/ | - | - | 0.007 |
| p-Chlorophenylchloroacetamide 10/ | 0.006 | - | 0.01 |
| Ammonium p-Chlorobenzenesulfonate 11/ | 0.005 | - | - |
| Sodium p-Chlorobenzenesulfonate 12/ | - | 0.02 | - |
| Inorganic Compounds | 0.01 | 0.1 | 0.04 |
| Unidentified Compounds and losses | 14.55 | 5.58 | 2.59 |

1/



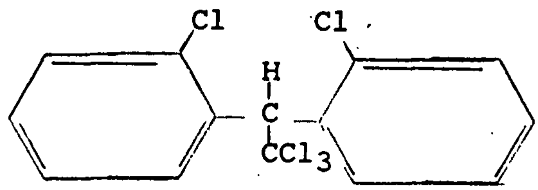

1,1,1-Trichloro-2,2-bis (p-Chlorophenyl)ethane

2/



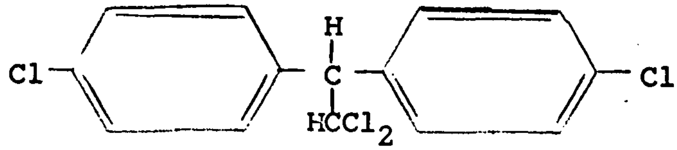
1,1,1-Trichloro-2-(c-Chlorophenyl)-2-(p-Chlorophenyl)ethane

3/



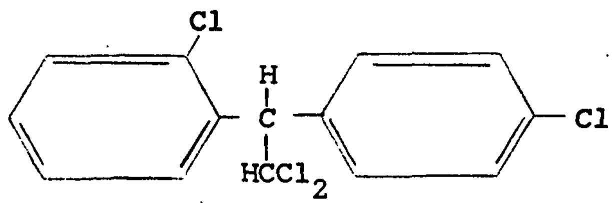
1,1,1-Trichloro-2,2-bis(o-Chlorophenyl)ethane

4/



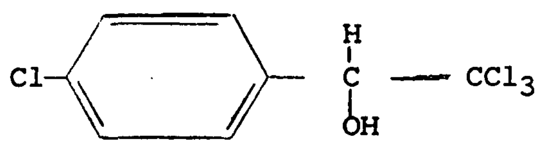
1,1-Dichloro-2,2-bis(p-Chlorophenyl)ethane

5/



1,1-Dichloro-2-(o-Chlorophenyl)-2-(p-Chlorophenyl)ethane

6/



p-Chlorophenyltrichloromethylcarbinol

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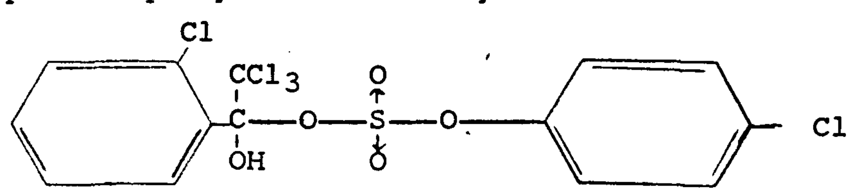
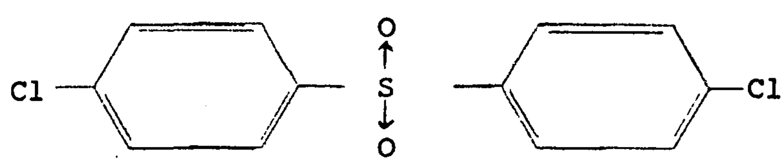


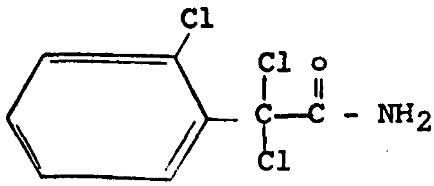
o-Chlorophenyltrichloromethylcarbinol p-Chlorobenzenesulfonate

8/



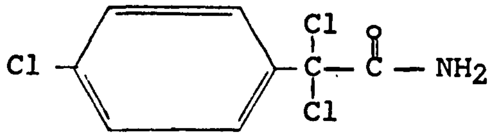
p,p'-Dichlorodiphenyl sulfone

9/



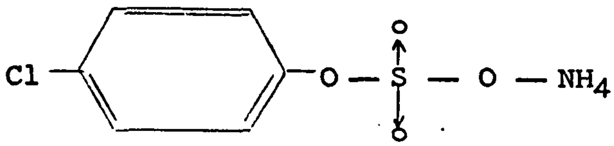
o-Chlorophenylchloroacetamide

10/



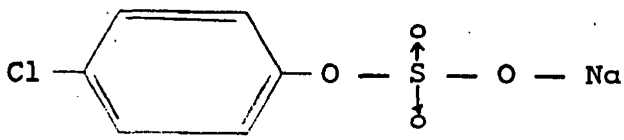
p-Chlorophenylchloroacetamide

11/



Ammonium p-Chlorobenzenesulfonate

12/



Sodium p-Chlorobenzenesulfonate

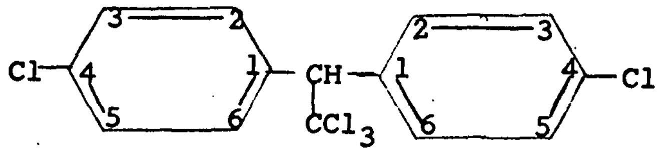
Of all the isomers of DDT only the p,p'-or the 4,4'-isomer has valuable insecticidal properties. The properties of the DDT isomer are given in Table 3.

Table 3

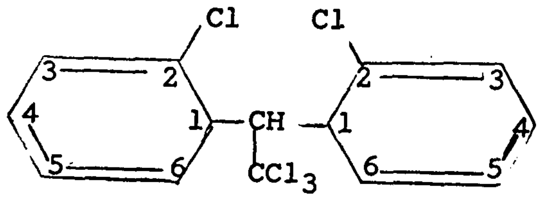
Insecticidal Properties of DDT Isomers

| Isomer | M.P. (°C) | Relative toxicity (house flies) |
|---|---|---|
| 4,4' 13/ | 108.5-109 | 1 |
| 2,2' 14/ | 92.5-93 | 0.011 |
| 2,3' 15/ | — | 0.015 |
| 2,4' 16/ | 74-74.5 | 0.018 |
| 3,4' 17/ | — | 0.9 |

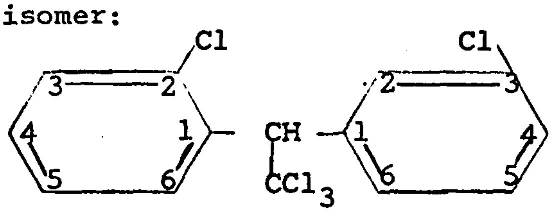
13/ 4,4' iomer:



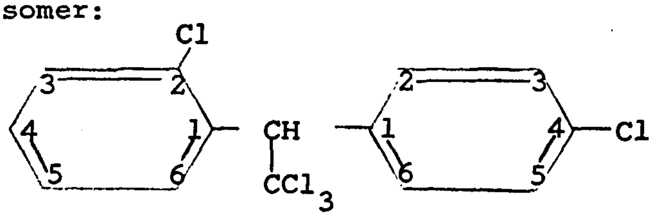
14/ 2,2' isomer:



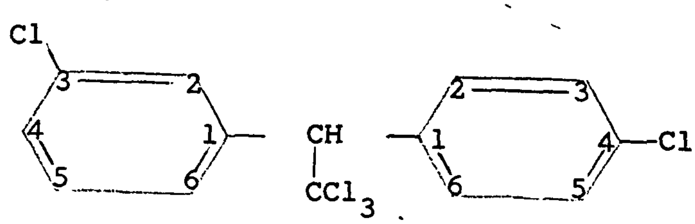
15/ 2,3' isomer:



16/ 2,4' isomer:

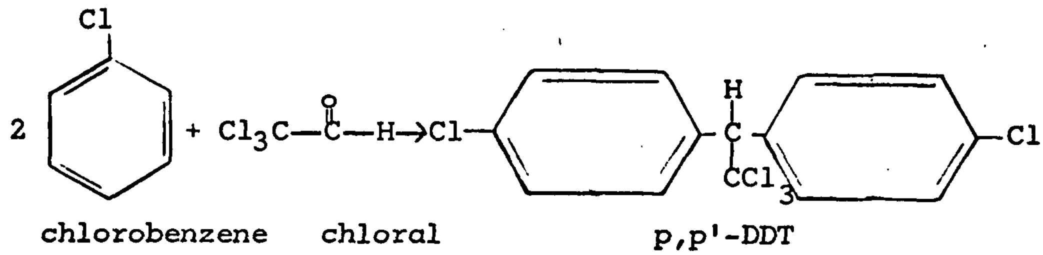


17/ 3,4' isomer:

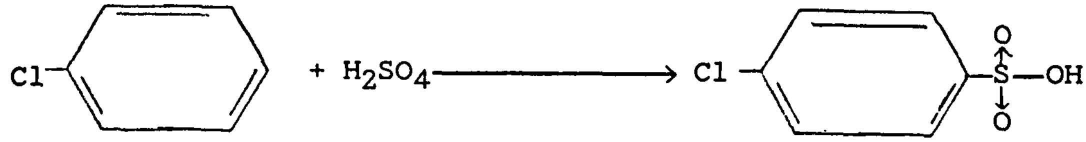


Because technical grade DDT has a much lower melting point than pure p,p'-DDT, it grinds poorly in ball mills, thereby hindering the preparation of wettable powders with a high content of the active ingredient. For preparing such powders, DDT without oily impurities is required. This is achieved by producing DDT from specifically purified chloral.

The principal method of producing DDT is the condensation of chlorobenzene with chloral:

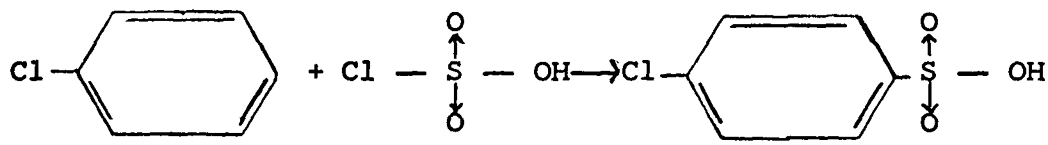



This reaction takes place in the presence of condensing agents such as concentrated sulfuric acid, oleum, chlorosulfonic acid, fluorosulfonic acid, hydrogen fluoride, anhydrous aluminum chloride, and others. In industry the most frequently used method is condensation of chloral with chlorobenzene in the presence of concentrated sulfuric acid or weals oleum at a temperature not higher than 20°C, since at higher temperatures the amount of p-Chlorobenzenesulfonic acid that is formed as a by-product increases sharply.




p-chlorobenzenesulfonic acid

Routes have now been developed for the use of p-Chlorobenzenesulfonic acid for the production of acaricides, DDT synergists, and other compounds. It also is possible to regenerate chlorobenzene from p-Chlorobenzenesulfonic acid in 95 percent yield by the action of dilute sulfuric acid at 200°-240°C. p-Chlorobenzenesulfonic acid is produced in a minimal amount when the condensation is carried out in the presence of chlorosulfonic acid. However, because of the high cost of chlorosulfonic acid this method is almost never used in industry.



Chlorosulfonic acid

Chloral is obtained by chlorination of ethyl alcohol or acetaldehyde. The chlorination of acetaldehyde proceeds through an enol form and can be represented by the following general equation:

$$3Cl_2 + \underset{\text{acetaldehyde}}{H_3C - \overset{O}{\overset{\|}{C}} - H} \longrightarrow \underset{\text{chloral}}{Cl_3C - \overset{O}{\overset{\|}{C}} - H} + 3HCl$$

The mechanism of chlorination of ethyl alcohol is more complex:

1. $CH_3CH_2OH + Cl_2 \longrightarrow [CH_3CH_2OCl]$
2. $[CH_3CH_2OCl] + HCl \longrightarrow CH_3\overset{O}{\overset{\|}{C}}-H + HCl$
3. $CH_3\overset{O}{\overset{\|}{C}}-H + Cl_2 + HOCH_2CH_3 \longrightarrow H_2\overset{Cl}{C}-\underset{H}{\overset{Cl}{C}}-O-CH_2CH_3 + H_2O$

4. $$CH_3\overset{O}{\overset{\|}{C}}\text{-}H + Cl_2 + 2HOCH_2CH_3 \longrightarrow H_2\overset{Cl}{\overset{|}{C}}\text{-}\overset{OCH_2CH_3}{\underset{OCH_2CH_3}{C}}\text{-}H \quad + HCl + H_2O$$

5. $$H_2\overset{Cl}{C}-\overset{OCH_2CH_3}{\underset{OCH_2CH_3}{C}}-H \quad + Cl_2 \longrightarrow H\text{-}\overset{Cl}{\underset{Cl}{C}}-\overset{OCH_2CH_3}{\underset{OCH_2CH_3}{C}}-H \quad + HCl$$

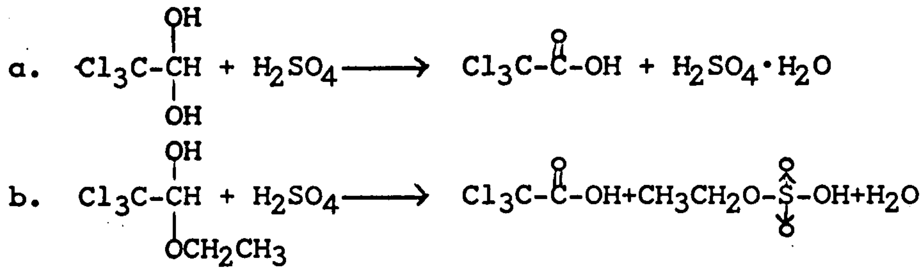
6. $$H-\overset{Cl}{\underset{Cl}{C}}-\overset{OCH_2CH_3}{\underset{OCH_2CH_3}{C}}-H \quad + H_2O \longrightarrow H-\overset{Cl}{\underset{Cl}{C}}-\overset{OH}{\underset{OCH_2CH_3}{C}}-H \quad + CH_3CH_2OH$$

7. $$H-\overset{Cl}{\underset{Cl}{C}}-\overset{OH}{\underset{OCH_2CH_3}{C}}-H \quad + Cl_2 \longrightarrow Cl\text{-}\overset{Cl}{\underset{Cl}{C}}-\overset{OH}{\underset{OCH_2CH_3}{C}}-H \quad + HCl$$

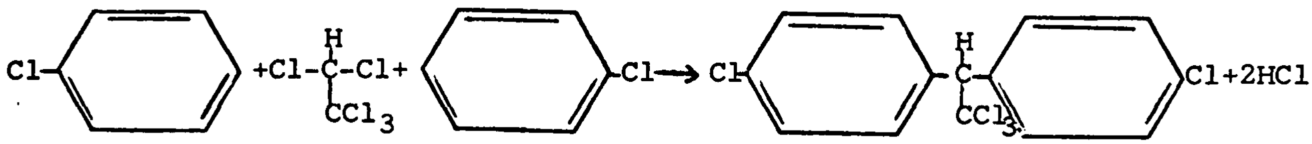
8. $$Cl_3C-\overset{OH}{\underset{OCH_2CH_3}{C}}-H \quad + H_2O \longrightarrow Cl_3C-\overset{O}{\overset{\|}{C}}-H + CH_3CH_2OH$$

It can be seen from the equations presented above that the chlorination of ethyl alcohol is best carried out in the presence of a small amount of water. It has been established, experimentally, that 14 kilograms of water to 100 kilograms of alcohol distillate is optimum. The process can be carried out as either a batch or a continuous process. The chlorination is carried out in the first stage at 50°-60°C, and then at 90°C.

The product obtained by the chlorination of ethyl alcohol contains chloral alcoholate and chloral hydrate, which on treatment of the reaction mixture with concentrated sulfuric acid go over to chloral:



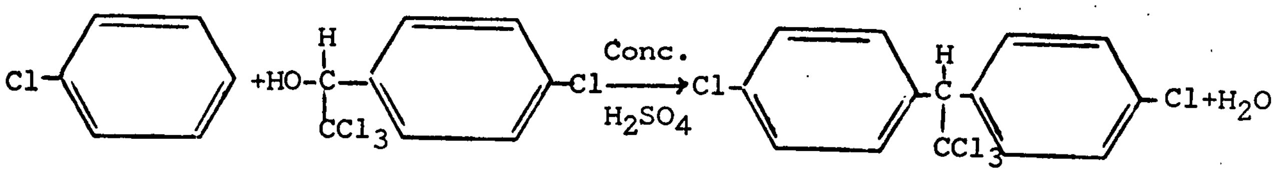
It is also possible to obtain DDT by the condensation of chlorobenzene with pentachloroethane:



pentachloroethane

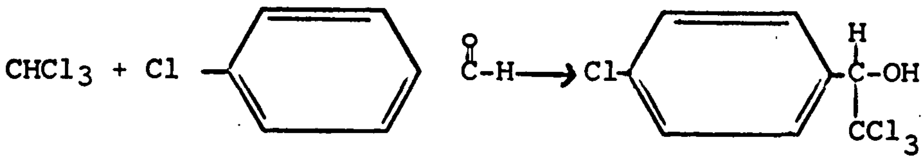
However, the DDT produced by this method is strongly contaminated with various by-products.

An interesting method for the synthesis of DDT and especially of its unsymmetrical anologs is the reaction of chlorobenzene with p-Chlorophenyltrichloromethylcarbinol:



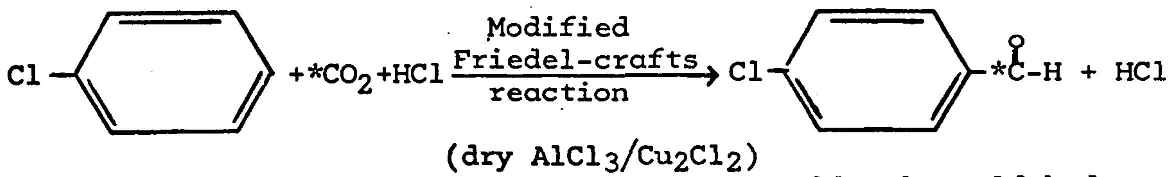

p-Chlorophenyl-
trichloromethylcarbinol

This reaction proceeds readily in the presence of sulfuric acid or oleum. The p-Chlorophenyltrichloromethylcarbinol is prepared from chloroform and p-Chlorobenzaldehyde:

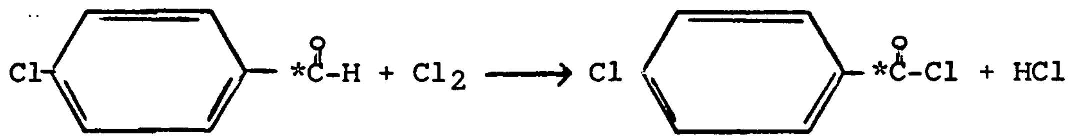


For the synthesis of the radioactive C-14 labeled DDT the following laboratory reactions may be employed:

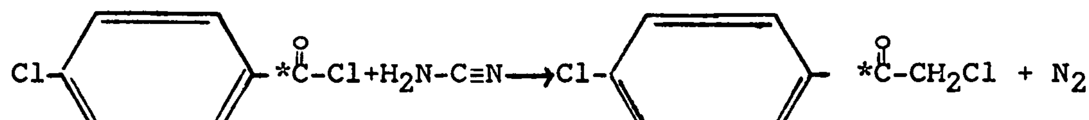
1. $*C + O_2 \longrightarrow *CO_2$ (*C denotes C-14)

2. 

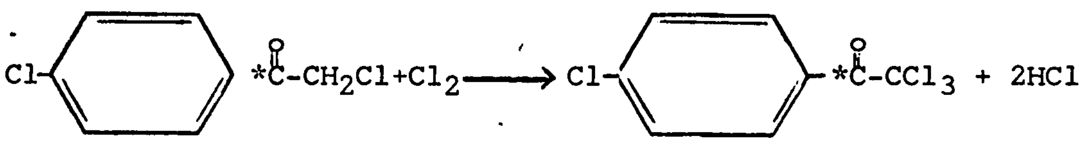
p-Chlorobenzaldehyde

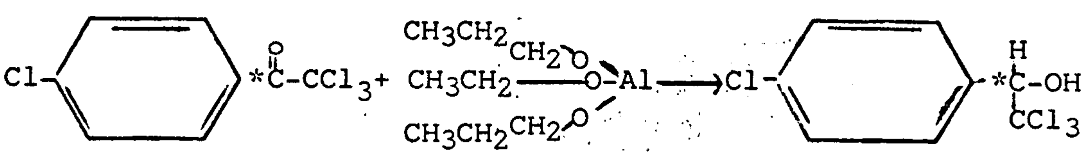
3. 

p-Chlorobenzoyl chloride

4. 

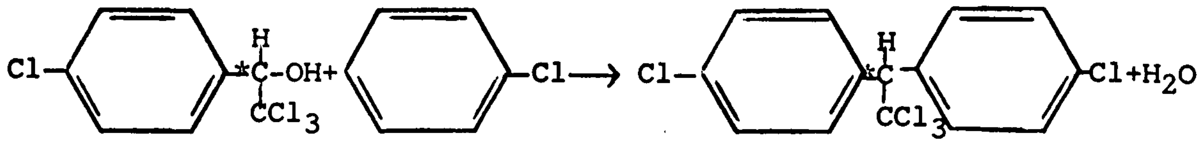
Cyanamide

5. 

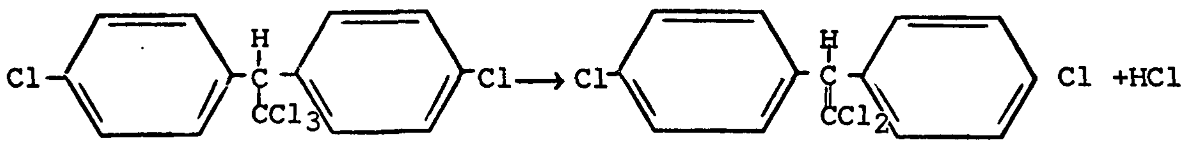
6. 

Aluminum propylate

p-Chlorophenyl-trichloromethylcarbinol

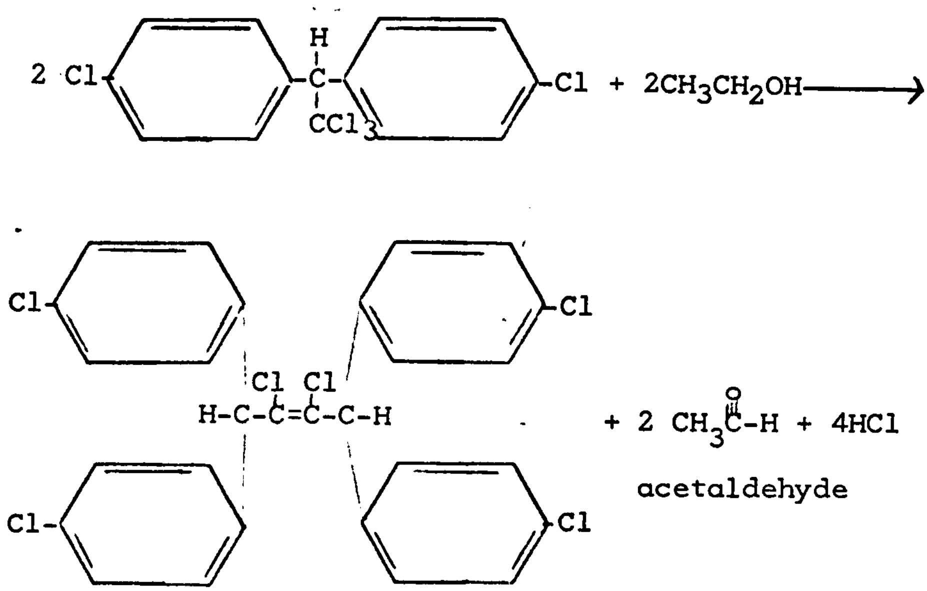
7. 

The pure p,p'-isomer of DDT is thermally stable. Its decomposition starts above 195°C and proceeds according to the equation:

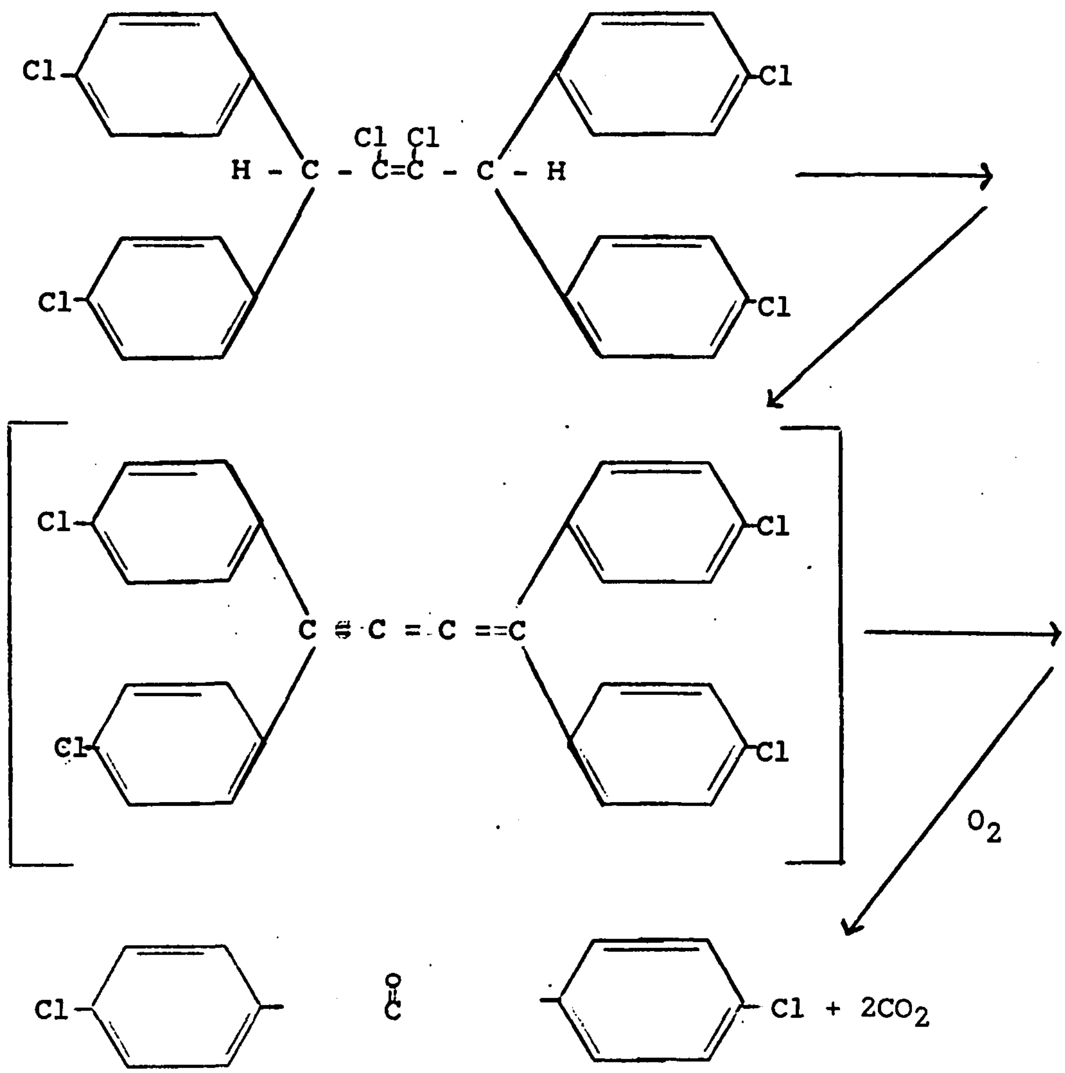


Iron salt impurities sharply lower the decomposition temperature of DDT. For example, in the presence of only 0.01 percent ferric chloride ($FeCl_3$), the decomposition temperature is lowered to 120°C.

When DDT decomposes under the influence of sunlight in alcohol solution, the following reactions take place:

1. 

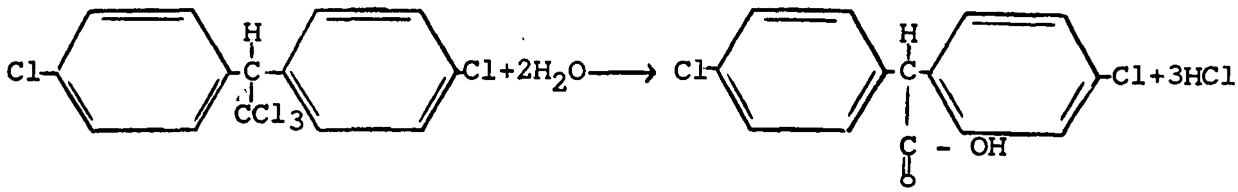
2. In the presence of the oxygen in air, the tetra, p-chlorobenzene compound above undergoes the following oxidation reaction:




p,p'-dichlorobenzophenone

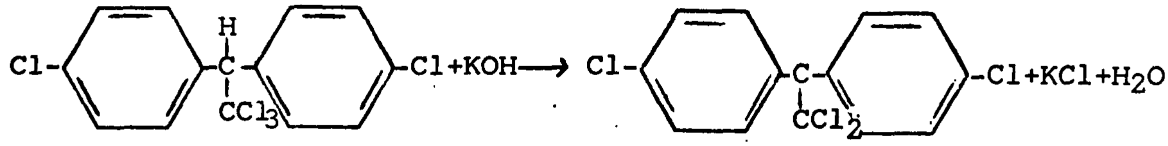
Apparently similar processes also occur on the leaves of plants.

Pure DDT at room temperature does not affect most metals, but technical preparations, especially those containing water and salt solutions, cause more or less corrosion. Probably this is associated with the evolution of HCl as a result of hydrolysis of the DDT by water as the following equation indicates:

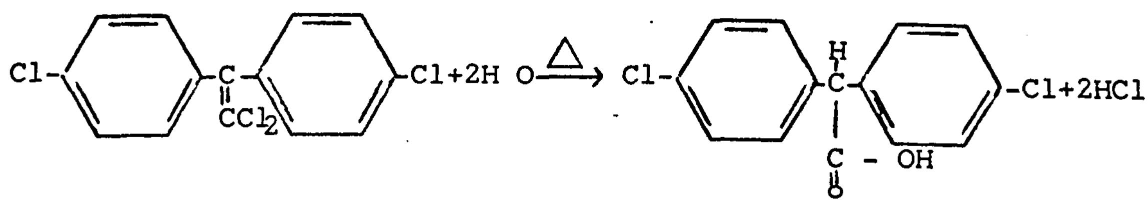


p,p'-dichlorodiphenylacetic acid

At room temperature, this reaction proceeds slowly, but when an aqueous suspension of DDT is boiled, the process is accelerated. Caustic alkalies, lime, barium hydroxide, and other alkaline agents increase the rate of hydrolysis of DDT. The first step of the reaction of DDT with alkalies is the splitting out of HCl and the formation of p,p'-dichlorodiphenylethylene, which further reacts at a higher temperature to p,p'-dichlorodiphenylacetic acid:

1. 

p,p'-dichlorodiphenylethylene

2. 

This reaction is employed for the quantitative determination of DDT and also of the p,p'-isomer of DDT in the technical grade product. Determination of p,p'-DDT is based on the different rate of splitting out of HCl by caustic alkalies from the isomers of DDT.

Extensive investigations have been carried out on the synthesis and on the biological activity of homologs and analogs of DDT. When chlorine in the aromatic radical of 1,1,1-trichloro-2,2-bis (p-Chlorophenyl) ethane is replaced by hydrogen (Fig. 1), bromine (Fig. 2), iodine (Fig. 3), hydroxyl (Fig. 4), a higher hydrocarbon radical (Fig. 5), amino (Fig. 6), thiocyano (Fig. 7), carboxyl (Fig. 8), nitro (Fig. 9), and cyano (Fig. 10) groups the insecticidal activity of the compound is substantially lowered. A lowering of the insecticidal activity also occurs when several methyl (Fig. 11) or alkoxy (Fig. 12) groups are introduced into the aromatic radicals.

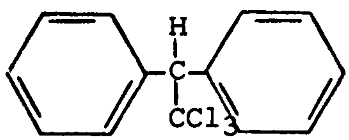



Figure 1

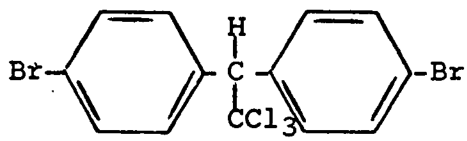



Figure 2

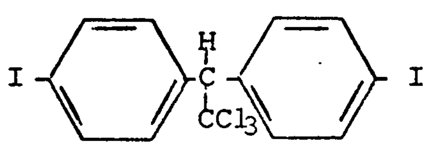



Figure 3

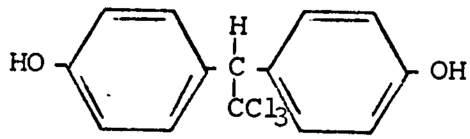



Figure 4

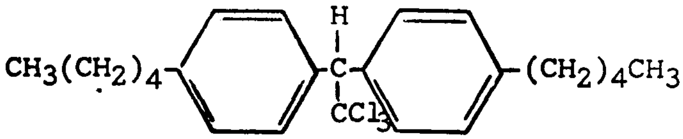


Figure 5

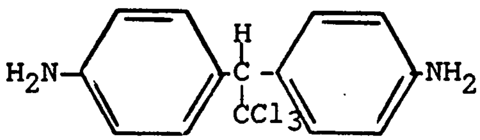


Figure 6

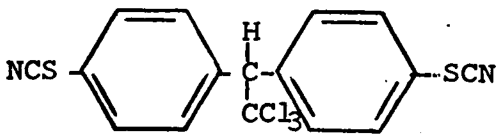


Figure 7

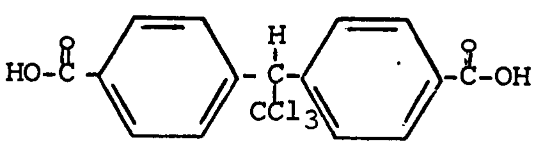


Figure 8

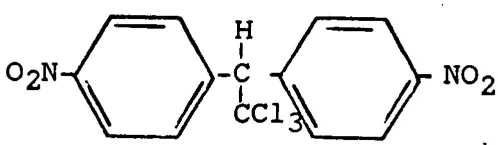


Figure 9

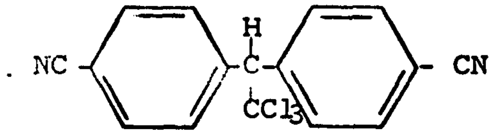


Figure 10

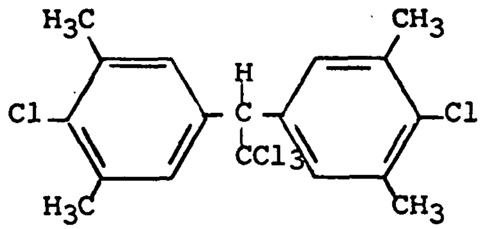


Figure 11

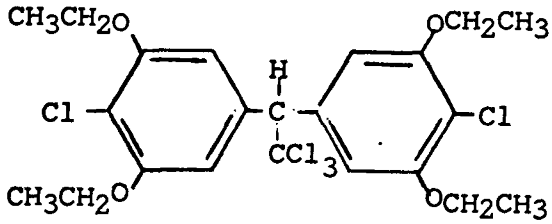


Figure 12

Replacement of chlorine by fluorine (Fig. 13), methoyl (Fig. 14), methyl (Fig. 15), or ethyl (Fig. 16), does not substantially change the insecticidal activity of the compound, but it lowers the toxicity for vertebrates and man.

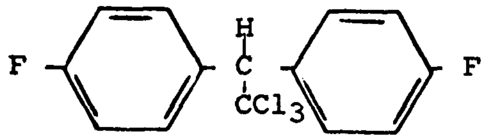


Figure 13

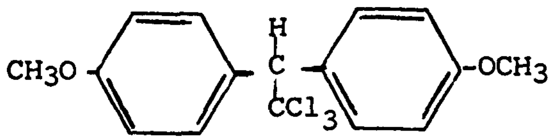


Figure 14

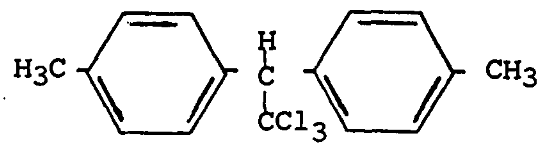


Figure 15

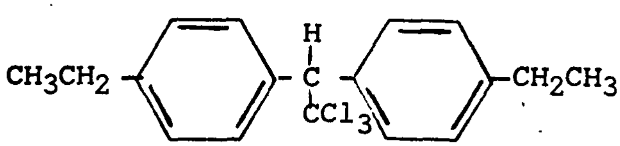


Figure 16

For examples, 1,1,1-Trichloro-2,2-bis (p-methoxyphenyl) ethane (Fig. 14) is one-fortieth as toxic as DDT for mammals, while 1,1,1-Trichloro-2,2-bis (p-ethoxyphenyl) ethane (Fig. 17) is two-thirds as toxic.

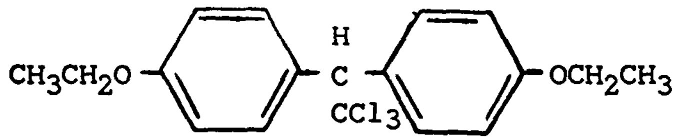


Figure 17

Trichlorodinaphthylethane (Fig. 18), trichlorodithienylethane (Fig. 19), and others are weak insecticides. The unsymmetrical analogs also, as a rule, are considerably less active than DDT.

Figure 18

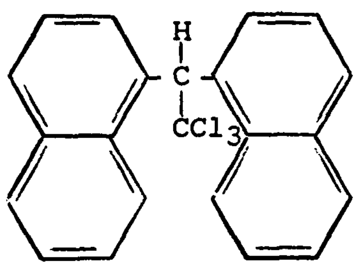
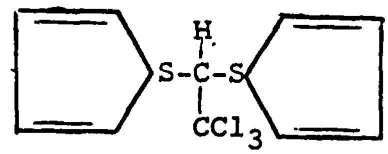


Figure 19



Removal of the trichloromethyl group from the aromatic radicals leads to less active compounds, as does also the splitting out of HCl from compounds containing the trichloromethyl group. Replacement of one chlorine (Fig. 20) in the trichloromethyl group by hydrogen lowers the insecticidal activity by two to four times, and the toxicity for animals by 5 to 15 times. When a second chlorine atom (Fig. 21) is replaced by hydrogen, a fivefold decrease in toxicity for animals is observed in comparison with DDT and the insecticidal activity decreases 5 to 50 times.

Replacement of chlorine by fluorine (Fig. 23), bromine (Fig. 24), alkyl groups (Fig. 25), and other similar groups in most cases leads to a substantial lowering of the insecticidal properties. p,p'-Dichlorodiphenylethane (Fig. 22) has practically no toxic effects toward insects, but it is an acaricide. The corresponding dichlorodiarylethylenes (Fig. 26) are also practically nontoxic toward insects. The diaryltrichlorovinylmethanes (Fig. 27) have only weak insecticidal properties.

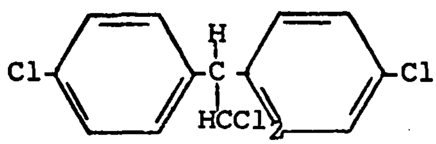


Figure 20

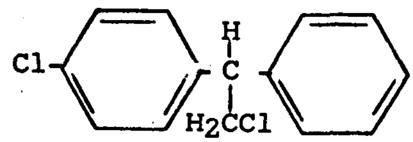


Figure 21

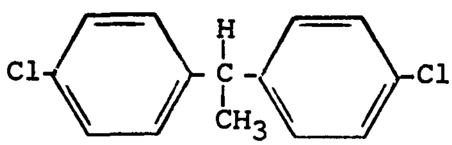


Figure 22

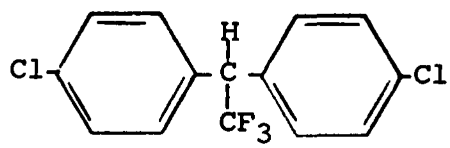


Figure 23

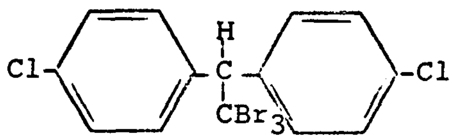


Figure 24

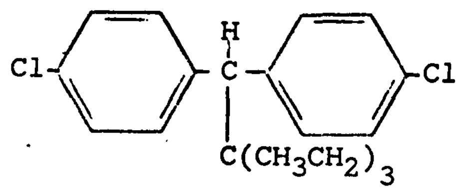


Figure 25

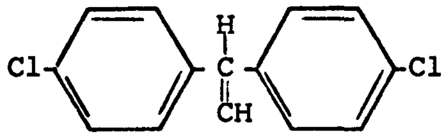


Figure 26

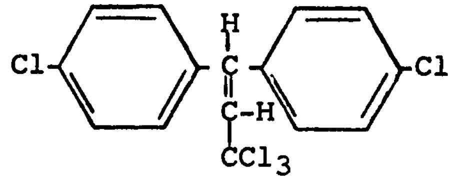
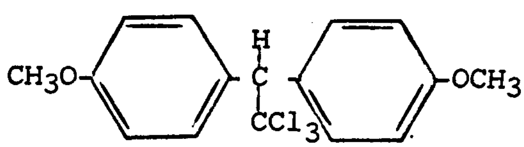
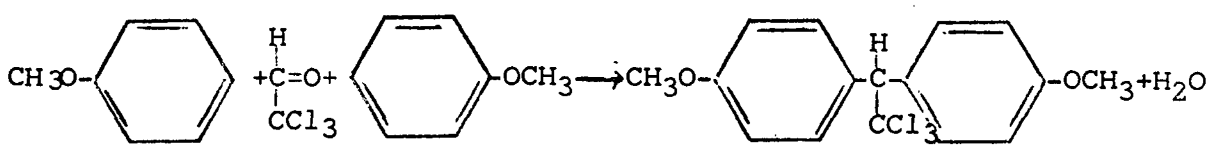


Figure 27

Of a large number of analogs and homologs of DDT that have been studied, a few, which are described briefly below, have found practical use. These analogs are commonly known as methoxychlor, DDD, Perthane, and DFDT. The official chemical name for methoxychlor is 1,1,1-trichloro-2,2-bis (p-methoxyphenyl) ethane. It has the following chemical structure:



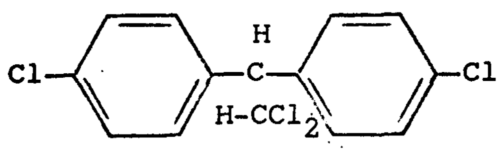
Methoxychlor is obtained in good yields by the condensation of chloral with anisole in the presence of sulfuric acid as indicated in the equation below.



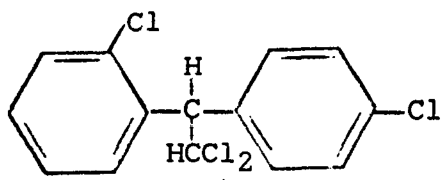
anisole chloral methoxychlor

In contrast to the manufacture of DDT, the use of oleum ($H_2S_2O_7$) is not recommended in making methoxychlor, because in this process a very large amount of sulfonation products is obtained. The technical grade product contains not less than 88 percent of the p,p'-isomer and a small amount of the o,p'-isomer. Methoxychlor has limited use because not only does it cost more than DDT, but it also has little effectiveness toward a number of insects. It often is contained in various praparations based on DDT and lindane as a third component. Methoxychlor formulations are completely similar to those of DDT.

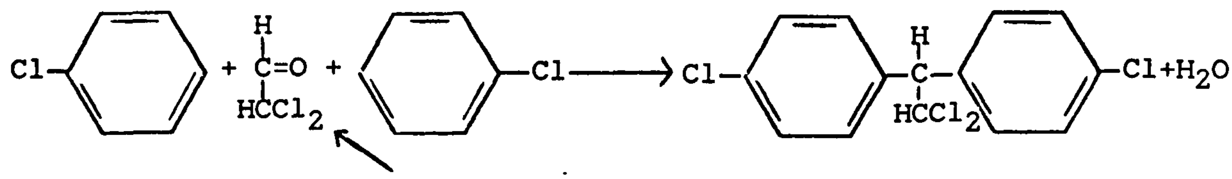
In addition to methoxychlor, DDD has found wide use in agriculture. DDD is also known as TDE (tetrachloro-diphenylethane). Its official chemical name is 1,1-Dichloro-2,2-bis (p-Chlorophenyl) ethane and has the following chemical structure:



DDD consists of white crystals with a melting point of 112°C. The technical grade preparation has a setting point of about 86°C and contains as the main impurity the o,p'-isomer; i.e.



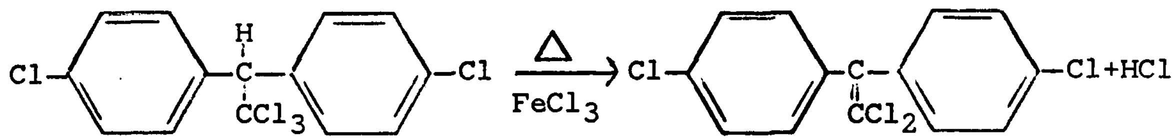
The o,p!-isomer has indicated promising results in the medical treatment of malignant tumors of the adrenal glands. DDD is one of the metabolites of DDT. It is produced by the condensation of chlorobenzene with dichloroacetaldehyde, which must be very pure and not contain chloral as an impurity. The reaction goes as follows:



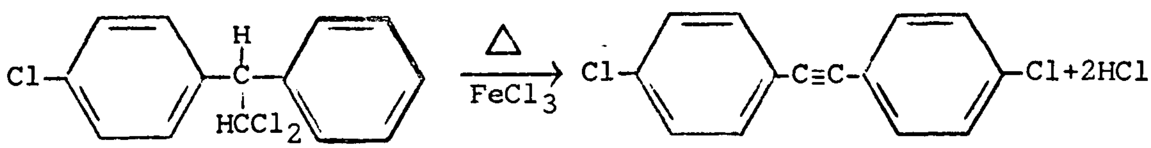

Chlorobenzene Dichloroacetaldehyde

DDD differs somewhat from DDT in its chemical properties. Where DDT splits out one molecule of HCl when treated with ferric chloride, DDD splits out two molecules of HCl. The respective equations are as follows:

a. DDT:



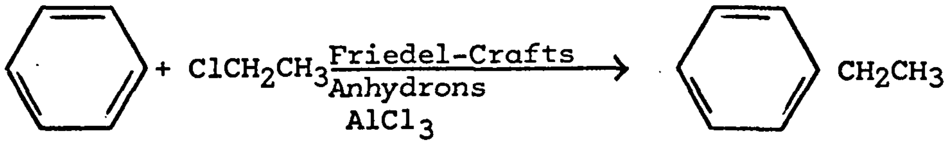

b. DDD:




Dichlorotolan

Perthane is a selective insecticide to control pests of stone fruits, lettuce, and spinach and also to control flies in animal husbandry. Perthane is a white crystalline substance with a melting point of 56°-57°C. It is insoluble in water, highly soluble in organic solvents. It is produced by the condensation of dichloroacetaldehyde with ethylbenzene in the presence of sulfuric acid:

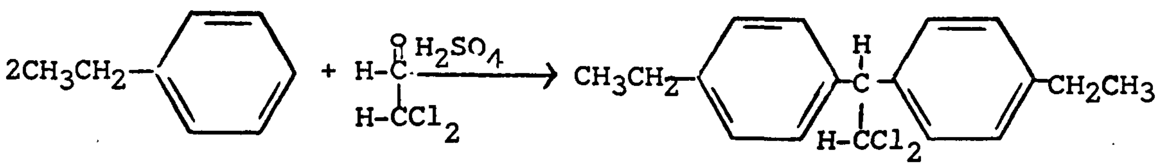
a.




ethylbenzene

b.

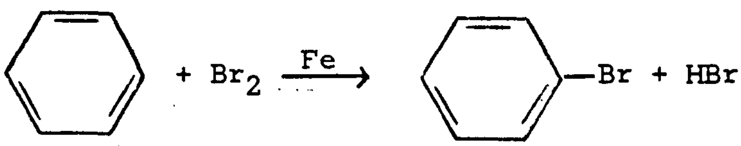
Perthane



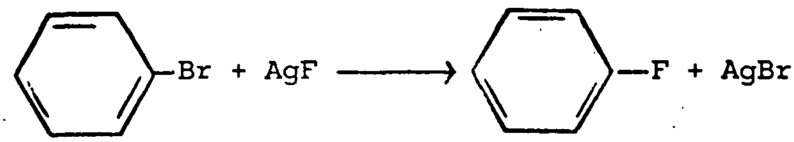

Perthane is one-fifth to one-tenth as active as DDT.

DFDT is another anolog of DDT. It has a melting point of 45°C. It is almost insoluble in water, but more soluble than DDT in organic solvents. DFDT is produced by the condensation of chloral with fluorobenzene in the presence of sulfuric acid.

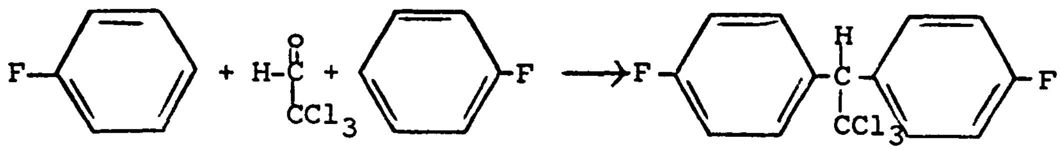
a.



b.



c.



DFDT

The official chemical name of the insecticide is 1,1,1-Trichloro-2,2-bis(p-fluorophenyl)ethane. DFDT is similar to DDT in chemical properties. Its toxicity for insects is close to that of DDT. The analog is less persistent than DDT, which in some cases is a great advantage. It is, however, more expensive than DDT because of the relatively high cost of fluorobenzene.

Metabolism of DDT:

There are five principal routes of DDT metabolism in various organisms:

1. Oxidation to DDA, common name for bis(chlorophenyl)acetic acid.
2. Oxidation to Kelthane, common name for 1,1-bis(p-chlorophenyl)-1,1,1-trichloro-ethanol
3. Oxidation to dichlorobenzophenone.

4. Dehydrochlorination to DDE, common name for dichlorodiphenyldichloroethylent.

5. Reductive dechlorination to DDD, common name for 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane.

Figure 28 gives the routes and the chemical structures of the metabolites of DDT.

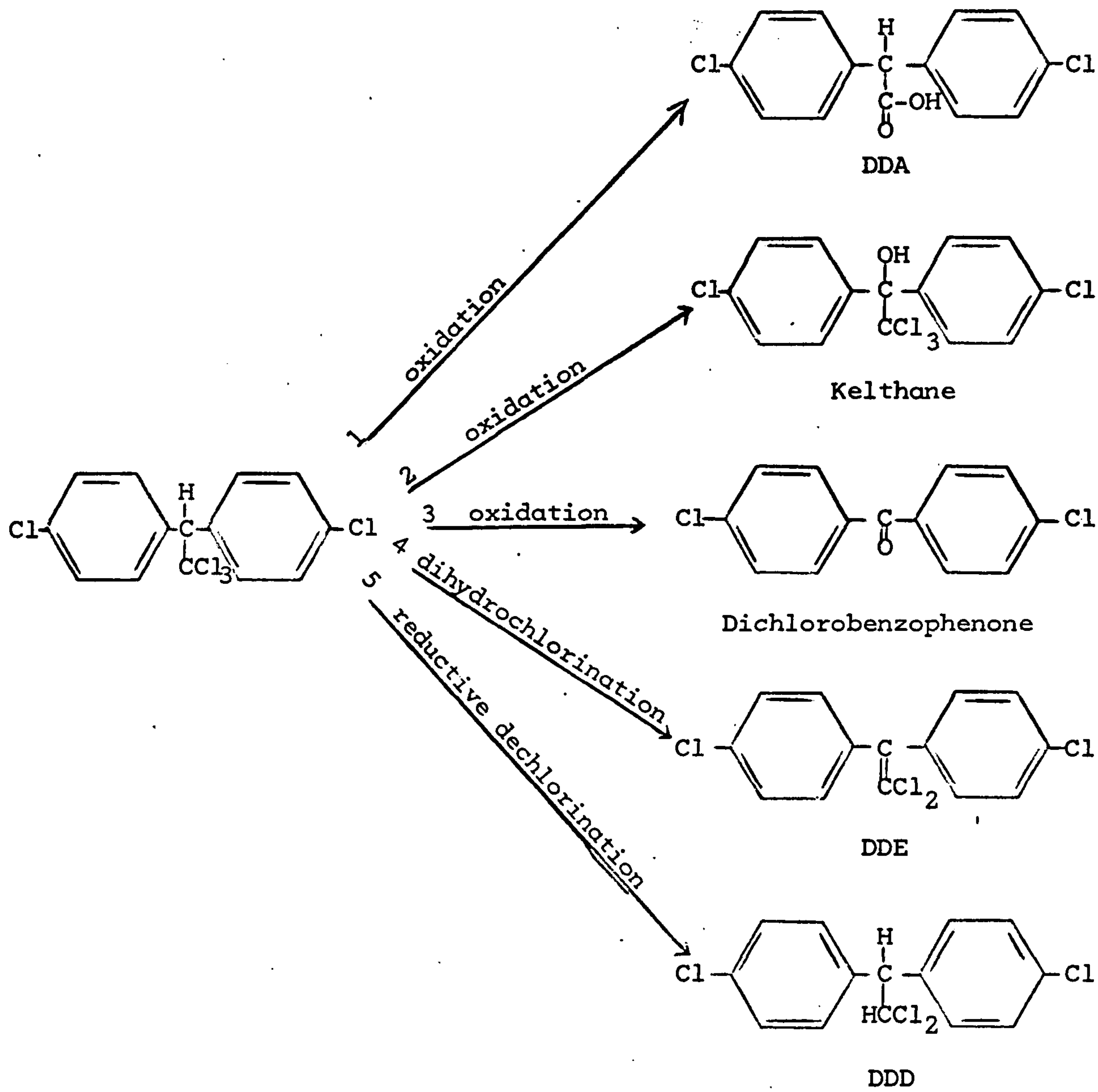



Figure 28. Routes of DDT Metabolism

In vertabrates, it has been known for almost 20 years that DDA, a common name for bis(chlorophenyl) acetic acid, is a major metabolite in feces and urine. Early reports claimed substantial amounts of unchanged DDT in urine and feces, but more recent findings have cast doubt on these reports, although there is no doubt that modest amounts of DDT occur in feces. Furthermore, the best study has shown that fecal DDA occurs as some derivatives whose nature is unknown; because DDA is produced from the derivative on acid, but not on alkaline, hydrolysis, it was suggested that the derivative might be an amide but was certainly not the usual glycoside. The pathway for DDA production in rats has recently been reported to involve alternate reduction and dehydrochlorination, followed by hydration and oxidation. This pathway is given in Figure 29.

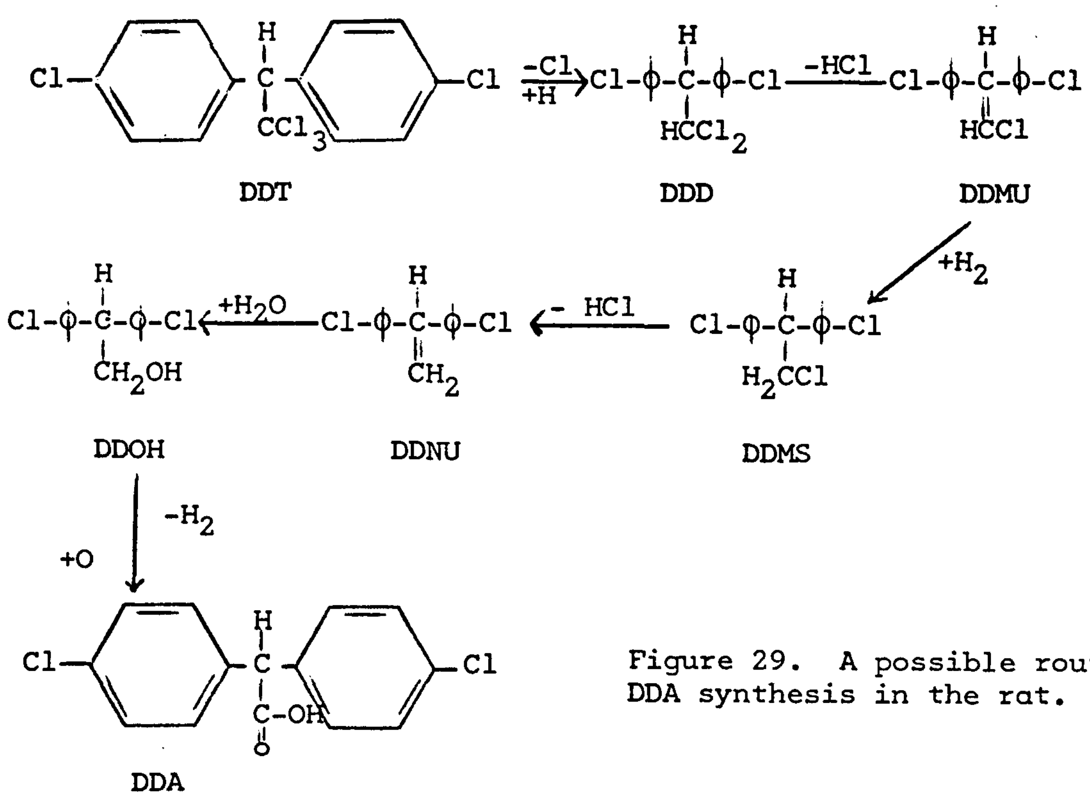



Figure 29. A possible route for DDA synthesis in the rat.

The evidence for this sequence is that the feeding of each compound to rats gave the subsequent intermediate as the major metabolite in liver. An exception is the last step, which is presumptive, for the metabolism of "DDOH" was not examined.

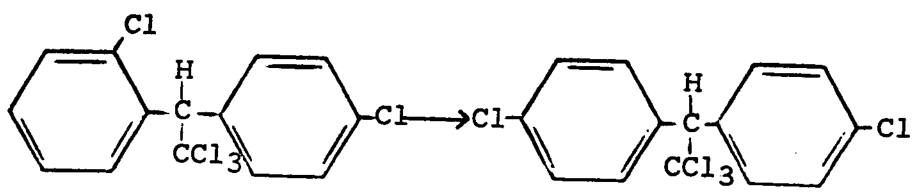
The simplifying view that DDA is the product of DDT metabolism in vertebrates is complicated by species variation. In man, DDE, a common name for dichlorodiphenyl-dichloroethylene, is the principal storage form of ingested DDT, and since DDE is not on the above route to DDA, that route is probably inoperative in man. By contrast, rats convert only small amounts of DDT to DDE, and monkeys none at all.

Only recently has it been realized that even in mammals another route of DDT metabolism is common; namely, reductive dechlorination to DDD, a common name for 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane. The reason for the delayed realization is that the standard Schechter-Haller method 1/ does not distinquish DDT from DDD; consequently, chromatographic techniques are required to separate them. Because DDD is insecticidal, this metabolism is not a detoxification. In 1963, it was reported that DDD was widely found in samples of water, soil, plants, and animal tissues obtained from

1/ The Schechter-Haller Method: /Schechter, M.S., Soloway, S.B., Hayes, R.A., and Haller, H.L. (1945). Ind. Eng. Chem., Anal. Ed., Vol. 17 (11), p. 704./ DDT is nitrated to the tetranitro derivative which produces a colored solution in benzene with a maximum absorption at 596 mμ when reacted with methanolic sodium methylate. However, any aromatic compound that may be nitrated is a potential source of interference. Homologs and analogs of DDT also respond to this analytical method.

areas where DDT, but not DDD, has been sprayed. In the same year, the reaction was demonstrated in yeast; and in 1965 in rats, mice, and rumen fluid. In the case of rats, it was found that DDD was present in the liver, but none was in body fat. In 1965, it was found that even lake water can convert DDT to DDD, and so can the two porphyrins, hematin and hemoglobin, in the reduced form. Since porphyrins are astonishingly stable with a half-life of billions of years in many media, it may be that the conversion in lake water utilizes porphyrins; and thus, the conversion reaction from DDT to DDD may not be enzymatically induced.

Isomerization in vivo is an unusual reaction for exotic compounds, but it has recently been shown that feeding o,p'-DDT leads to substantial residues of p,p'-DDT in the liver of rats.

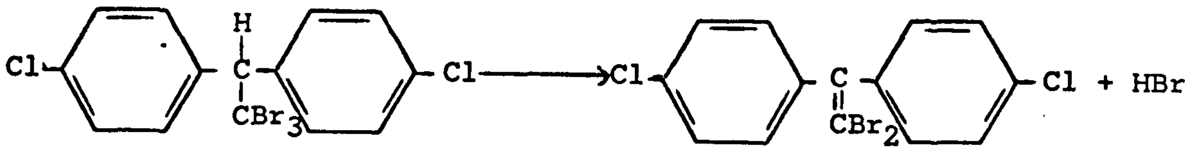


o,p'-DDT p,p'-DDT

Although there are other metabolities, the p,p'-DDT isomer was a major one. The parts per million (ppm) of DDD, p,p'-DDT, o,p'-DDT, and DDE were, respectively, o.64, 0.47, 0.12 and 0.10 after feeding 50 ppm of o,p'-DDT. The reverse isomerization of p,p'-DDT to o,p'-DDT is of very small, perhaps zero, importance. In seven experiments in which p,p'-DDT was fed at 50 ppm, the o,p'-DDT was found only in two cases and at low levels: 0.09 and 0.03 ppm.

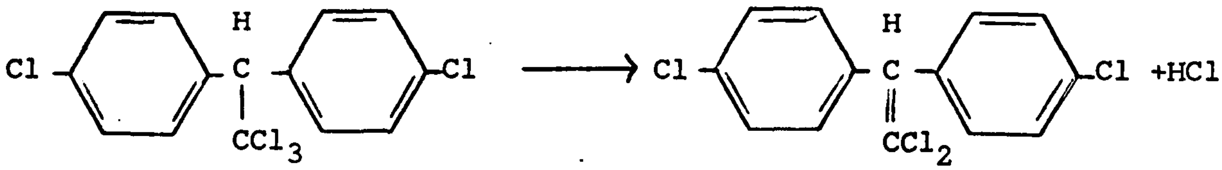
In insects, the best known metabolite of DDT is DDE. The ability of the housefly to dehydrochlorinate DDT to DDE may be a major cause of the exposed groups resistance to the DDT. The enzyme responsibel for this reaction is DDT-dehydrochlorinase. The enzyme has been greatly purified and its properties extensively studied. It exists, in very small concentrations, in susceptibel as well as in resistant houseflies. The enzyme is of moderate specificity, in that it also dehydrochlorinates DDD, but it is ineffective against o,p'-DDT. The Mexican bean beetle, which is normally tolerant to DDT and related compounds such as DDD and methoxychlor, has a relatively high titer of DDT-dehydrochlorinase. The level of this enzyme, measured by DDD dehydrochlorination, fluctuates during the development of the beetle, and this fluctuation is mirrored by parallel fluctuations in DDD tolerance.

Work with homogenates of houseflies, rather than with purified enzyme, has suggested that there may be more than one dehydrohalogenase enzyme. This is indicated by the fact that susceptible houseflies with little ability to dehydrochlorinate DDT can dehydrobrominate the $CBr_3$ analog excellently.



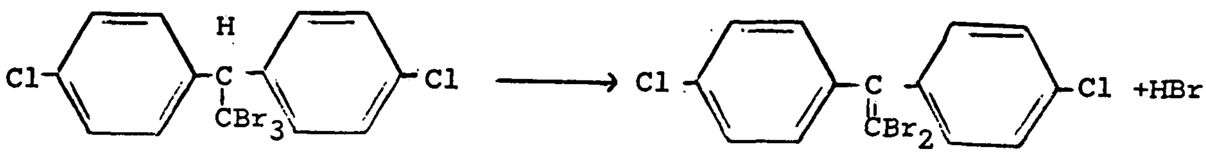
Studies on homogenates of resistant houseflies show that the $CBr_3$ analog is dehydrobrominated four times faster than DDT; the $CHBr_2$ or CHClBr, 15 times faster; and the $CHCl_2$ analog at one-thirtieth the rate.

1.



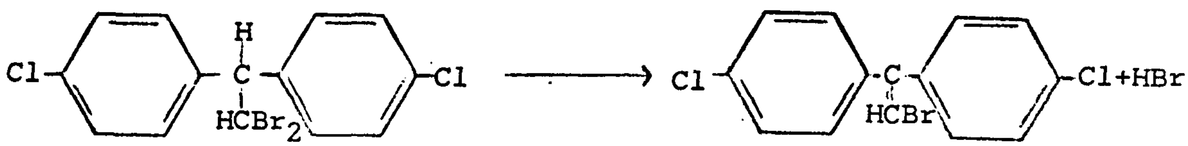
Relative rate = 1

2.



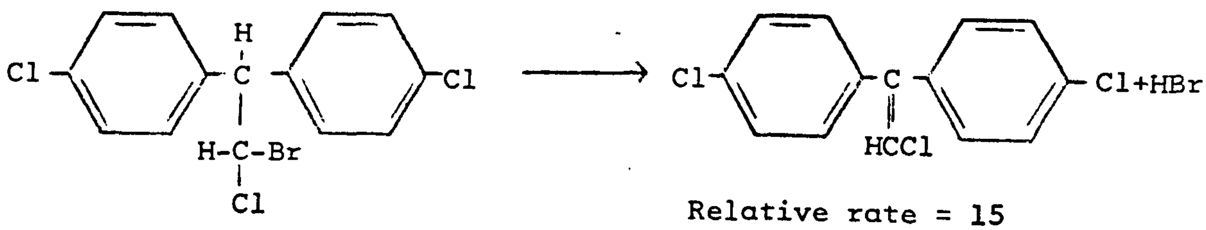
Relative rate = 4

3.



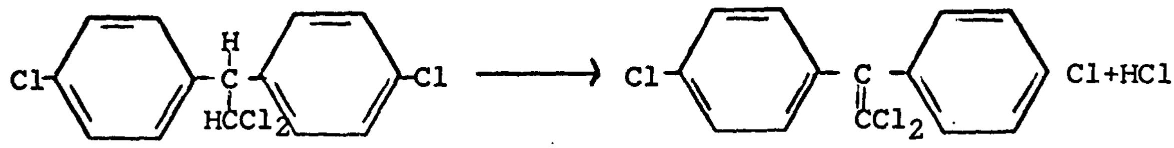
Relative rate = 15

4.



Relative rate = 15

5.



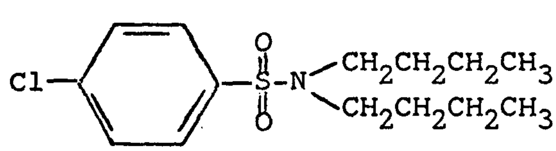
Relative rate = 1/30

In view of the above suggestion of the existence of more than one dehydrohalogenase, these findings may not describe the specificity of a single enzyme, but rather suggest that, in resistant insects too, more than one such enzyme exists.

Degradation to DDE is also a major pathway in some insects other than houseflies, including Mexican bean beetle, pink bollworm, and Aedes aegypti mosquito.

It seems that in resistant pink bollworms and in Aedes, as in the housefly, the resistance is primarily due to a far greater titer in resistant than in susceptible strains. However, the DDT dehydrochlorinases may differ in different species. In the resistant housefly, the enzyme is specific enough that o,p-DDT is not dehydrochlorinated, so that resistant insects can be killed by this compound.

An inhibitor for DDT-dehydrochlorinase has been developed; i.e., "WARF antiresistant." (WARF are the initials of the Wisconsin Alumni Research Foundation). Its chemical name is N,N-Dibutyl-4-chlorobenzenesulfonamide. It has the following chemical structure:



This compound has the ability to synergize the toxicity of DDT to resistant houseflies and Aedes, and is therefore considered to operate by blocking DDT-dehydrochlorinase.

It was found that DDA, originally thought to be primarily a metabolite in vertebrates, is also important in at least one insect; body house. In the homogenates of this insect, DDT is degraded to DDA, dichlorobenzophenone, and DDE in the ratio 2:2:1. The enzyme (or enzymes) involved showed astonishing heat stability, for it could be boiled for an hour withour loss of activity! Fractionation suggested that at least two enzymes were involved.

In the domestic fruit fly Drosophila melanogaster, quite different metabolic routes exist. In 1959, an investigator showed that there was quite extensive matabolism to Kelthane. Later studies made with $C^{14}$-DDT showed that dichlorobenzophenone is the other major metabolite in the larvae. In adult fruit flies, two other principal metabolites were demonstrated without being identified. In this study, a remarkable strain variation was found; Kelthane was the major metabolite in strain Oregon Rc, but was not a metabolite in strain Oregon R. An enzyme system for converting DDT to Kelthane, or a Kethane-like material, has been shown in microsomers from German cockroaches.

<u>Mechanism of Action</u>

It is painful to have to admit that, after decades of intensive research, there is still much to be desired in the full explanation of the mechanism of action of DDT. It is, however, generally agreed now that the insecticide's primary effects are virtually all upon the nervous system, both in vertebrates and invertebrates. The evidence for this view is as follows:

(1) The symptoms of poisoning suggest nervous impairment. In the American cockroach, for example, there is tremor throughout the body and appendages. These symptoms are commonly called "DDT jitters". Additionally, the treated insect showed hyperexcitability, followed very slowly by loss of motion or ataxia and total paralysis within 24 hours. In more sensitive insects, such as houseflies, fruit flies, and bees, the symptoms are similar but appear more rapidly, with paralysis in a few hours. Similarly, in mammals there is hyperexcitability and tremor, which is particularly evident in the face, and later there are convulsions, which may be both tonic, (i.e., the animal is rigid) with opisthotonus (the animal's head is arched back) and clonic (the animal is frenzied with uncoordinated movements). Finally, there is weakness and prostration.

(2) When DDT is applied to isolated tissues and enzymes, only nervous tissue is sensitive to very low concentrations. This statement is true only if one

excludes those effects given equally by DDT and nontoxic analogs, such as DDE.

(3) In the DDT-treated rat, an excellent correlation has been found between the level of DDT in the central nervous system and the intensity of symptoms.

On the above evidence it seems safe to conclude that DDT is a neurotoxicant. Two questions are begged from this conclusion; namely, 1. Where does the disruption of the nervous system occur? 2. What is the explanation for the disruption?

In 1945, a group of investigators showed the now familiar physiological symptoms of DDT poisoning of the nerve. According to these investigations, the phenomenon was a multiple effect; i.e., single nerve impulses arriving at a DDT-treated region of the nerve give rise to prolonged volleys of impulses. These volleys may account for the "DDT-jitters" symptoms. This was demonstrated in crayfish, crabs, lobsters, and cockroaches. Cockroach nerve, however, was at least ten times less sensitive than the crustocean, a fact that paralleled the 40-fold lower toxicity of DDT to whole cockroaches than to whole crayfish. The multiple effect could also be demonstrated on isolated nerve trunk. Therefore, it may be reasonable to assume that DDT acts on axonic rather than on synaptic transmission, a property shared by very few drugs, the best known of which is veratrine.

The question of the relative sensitivity of various nerves to DDT has provoked different answers. Some investigators claimed that the motor nerves were more sensitive than the sensory ones. Others claimed the opposite to be true. It appears that the latter may be the case from investigations designed specifically to settle this moot point. In 1946, one investigation showed definitely that although 1,000 ppm of DDT in solution could affect motor nerves and even muscle fibers in the American cockroach, low concentrations in the order of 0.01 ppm have no effect on these, or on the central nervous system, but only upon sensory nerves. These findings were later confirmed by another investigation. It therefore seems extremely likely that DDT is lethal because of its effects on sensory nerves.

This conclusion, in turn, begs the following question: What physiological mechanism gives rise to this effect? An early speculation was that calcium ion permeability of the nerve was reduced, because the investigators' work in crayfish showed that lowering the calcium ion concentration antagonized the DDT effect. However, these calcium ion effects are not seen in insect nerve; and, therefore, probably have no connection with the poisoning of insects.

The most revealing studies on the mechanism of DDT excitation were begun in 1957 by a group of Japanese investigators, working with the crural nerve of the American cockroach, and using intracellular electrodes, which show the response of a single neuron rather than responses of bundles of nerves. The results of the investigations indicated that DDT affected the action potential in a specific way; namely, it increased the negative after-potential (NAP). Because the NAP is associated with potassium ion efflux in cockroaches as well as vertabrates, the Japanese investigators suggested that DDT specifically inhibits this efflux.

For reasons of clarity, it is well, perhaps, to digress briefly and discuss the neurobiological aspects of this particular phenomenon of potassium ion efflux and impulse transmission in nerves.

There are two quite different modes of nerve impulse transmission in the nervous system: axonic transmission which conveys an impulse from its arrival point, then along the axon to the meeting place with another cell, which may be another neuron or may be a muscle, gland, or sensory receptor cell. Across the junction between cells, synaptic transmission occurs. The term synapse was formerly used for junctions between two neurons, but is now generally used for junctions of neurons with other cells, even for the junction between neuron and muscle which has the specific name of neuromuscular junction.

Neurophysiologists used to be either "sparks" men or "soup" men: the sparks men believed all transmission was electrical; the soup men argued that it was chemical. In fact, it is now firmly established that virtually all axonic transmission is "electrical", in a manner of speaking, and virtually all synaptic transmission is chemical.

The modern understanding of the basis of axonic transmission is due largely to A.L. Hodgin and A.F. Huxley. If one pokes an electrode into a resting axon, and measures the internal potential of the axon with respect to some outside point, the inside is found to be more negative than the outside; i.e., the axon is polarized. The resting potential difference is the "membrane potential." When a nerve impulse goes by, the inside suddenly becomes more negative than the outside, but recovers as the impulse passes on. In fact, it is this propagated reversal of polarity that constitutes the impulse. This moving deploarization is called an action potential.

The resting potential is believed to be caused by the existence of a higher potassium ion level inside the nerve than outside. On the other hand, the sodium ion level is higher on the outside portion of a nerve's membrane. The first or rising part of the action potential is caused by the sudden development of leakiness to sodium ions in the axon's outer membrane, so that sodium rushes in and the potential rapidly drops to zero and even goes positive. These potentials are commonly called after-potentials, either negative or positive. Then, microseconds later, the membrane becomes leaky to potassium ions that, because their concentration is higher inside than out, rush out and restore the equilibrium. It is further believed that a system, picturesquely called the sodium pump, is always busy pumping sodium ions out of the nerve to maintain its low internal sodium ion concentration, and hence the nerve's ability to be fired. The causal relationships to explain the sudden sodium ion leakiness in the membrane is unanswered.

To return to the experiments done in Japan, the investigators showed that high potassium concentrations reduced the effects of DDT on cockroach nerve, and low potassium concentrations enormously enhanced them, so that the negative after-potential (NAP) in a potassium-free environment appeared as a plateau rather than as a shoulder as given in Figure 30.

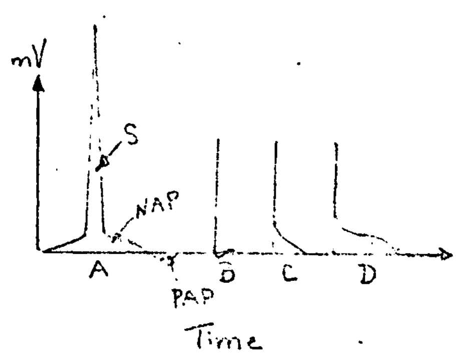



Figure 30. Action potentials recorded intracellularly.

A. A schematic diagram (S=spike, NAP=negative after-potential, PAP=positive after-potential).

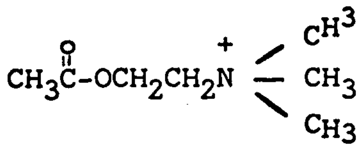
B. Drawing based on normal cockroach giant axon data.

C. 100 minutes after $10^{-4}$ M DDT.

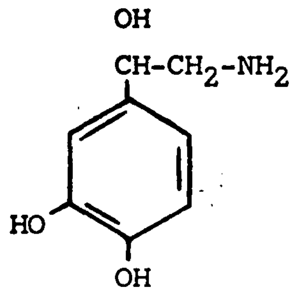
D. Same in potassium-free solution.

In addition to the "potassium-sodium" ions exchange explanation above, there is also a molecular basis for the physiological disruption in impulse transmission caused by DDT.

For a clearer understanding of this concept, a few words concerning the chemistry of impulse transmission at the synaptic juncture may need to be said at this point. When an impulse, propagated along an axon, reaches a synapse, the impulse itself dies out. However, it causes release, from the end of the axon, of a little burst or quantum or cloud of a chemical, the transmitter substance, which diffuses across the synapse and triggers off another action potential if the synapse is between neurons, or an appropriate response if the synapse is between a neuron and some effector, such as muscle or gland. There are two known kinds of transmitter substance; namely, acetylcholine and norepinephrine. The chemical structures of these substances are given below.



acetylcholine



norepinephrine

Synapses which utilize acetylcholine are called cholinergic, those that utilize norepinephrine are called adrenergic.

The above explanation assumes that the transmitter substances stimulate some component on the far side of the synapse, or the post-synaptic side. This component is called the receptor. One explanation is that the transmitter substance combines with the receptor to produce a configurational change which alters the sodium ion permeability at that point, thus triggering an action potential or appropriate response. A diagram of a synapse is given in Figure 31.

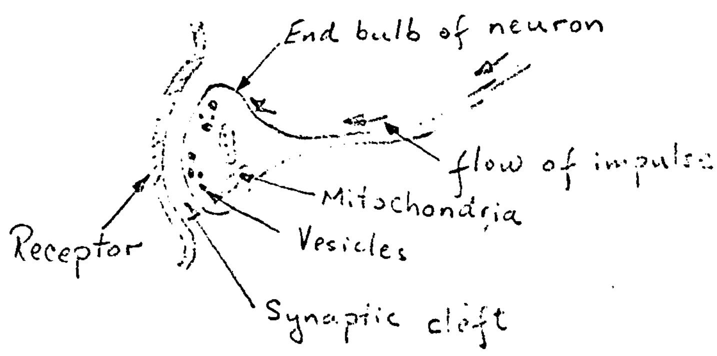


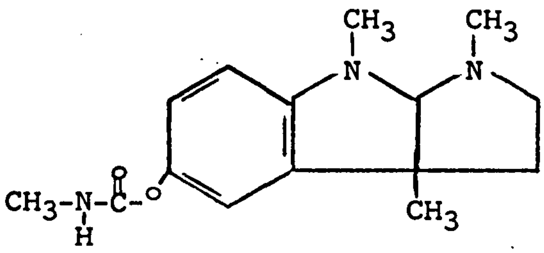

Figure 31. The components of a synapse

In order to restore the sensitivity of the synapse, the transmitter substances must be eliminated so that the receptor can return to its resting condition. At cholinergic junctions, acetylcholine is very promptly removed by cholinesterase, which hydrolyzes it to inactive components; i.e., acetic acid and choline. The reaction is as follows:

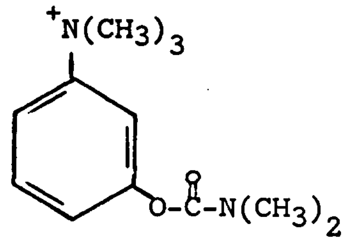
$$CH_3\overset{O}{\overset{\|}{C}}-OCH_2CH_2\overset{OH}{\overset{|}{N}}(CH_3)_3+H_2O \xrightarrow{\text{cholinesterase}} CH_3\overset{O}{\overset{\|}{C}}-OH+HOCH_2CH_2\overset{OH}{\overset{|}{N}}(CH_3)_3$$

The cholinesterase is commonly on the presynaptic side of the synapse rather than immediately adjacent to the recptor, but since the synaptic cleft, the zone between the presynaptic and postsynaptic endings, is only about 500 angstroms (Å) wide ($1Å=10^{-8}$ cm.), and each acetylcholine molecule is about 9 Å long, it is not difficult to imagine that presynaptic cholinesterase could rapidly eliminate acetylcholine throughout the synaptic cleft. In adrenergic junctions, the corresponding degrading enzyme is monoamine oxidase, but its precise localization is not known, and the current view is that diffusion away from the site is the major mode of loss, followed by oxidative removal at a relatively slow pace.

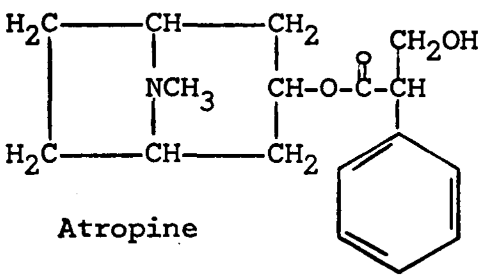
The enzymatic hydrolysis of acetylcholine is strongly inhibited by the alkaloids physostigmine, neostigmine, and atropine at levels as low as one part per million with respect to the inhibitor. The esterases are irreversibly inhibited by diisoprpylfluorophosphate. Chemical structures for these compounds are given below.



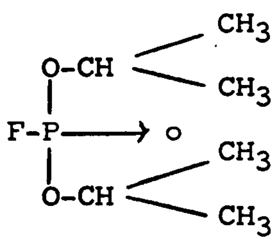
Physostigmine



Neostigmine



Atropine



Diisopropylfluorophosphate

Returning to the discussion of the molecular basis for the physiological disruption in nerve caused by DDT, there are several theories, which overlap a good deal, that are based upon the formation of some sort of complex between DDT and "the nerve membrane." By this, it is generally meant that the axonic membrane is involved in the action potential.

The results of one investigation demonstrated the strong affinity of DDT for cholesterol, which is common in nerve tissue, and speculated that combination with some important nerve-cell lipoids might cause "a state of excitability." Two attempts have been made to put this concept on a more specific footing. One attempt was made by L.J. Mullins in 1955. Mullins theorized that the target for lindane and DDT was a hypothetical lattice in the nerve membrane. This is a steric conception, comparable to that part of theorectical organic chemistry in attempting reaction mechanisms.

Mullins' hypothesis stemmed from his observations that certain isomers of 1,2,3,4,5,6-Hexachlorocyclohexane (HCH), popularly and chemically incorrect-called "benzene hexachloride" or "BHC", had little or no insecticidal effects on the target organism, while others were quite potent. He attempted to explain the marked variation in neural activity caused by the various HCH isomers on the basis of varying abilities to "fit" into a hypothetical lattice in the axonic membrane. He suggested that one might consider the lattice to resemble the spaces seen when one looks, end-on, at a pile of cylinders packed in the tightest possible way, which would be a hexagonal array (see Figure 32).

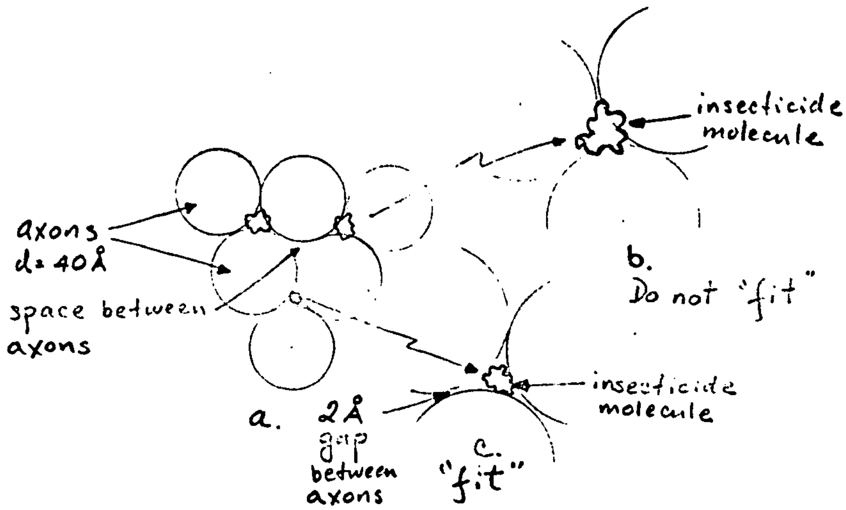
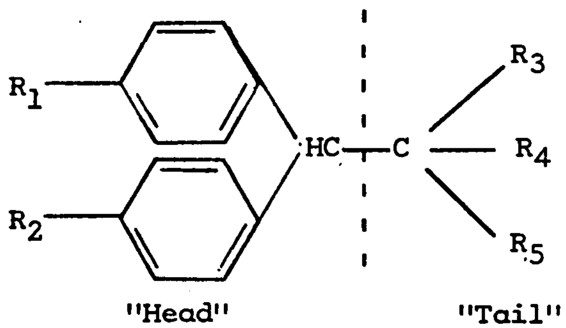



Figure 32. Mullins' model of an insecticide molecule in the axonic membrane lattice. The space is formed from a gap between three cycliders of 40Å diameter, separated from each other by 2Å.

The spaces are the hypothetical pores in the membrane lattice, and Mullins suggests that compounds are excitatory if they fit tightly into these pores. Compounds that are too large should have no effect. Compounds that can fit snugly might well distort the membrane structure and produce excitability. Compounds that are small enough to enter, but can only bind at one or two places of contact, would block the pores but not distort the membrane, and thus lessen excitability. Mullins hypothesized that if one considered a lattice made up of axons with diameters of 40 angstroms (Å) and with a separation gap of 2Å, the resultant spaces or pores made up of adjacent cylindrical axons were such that in the plane orientation - that is, pushing the HCH isomer in with its flat face flushed with the plane of the pore -, the gamma HCH isomer fitted neatly. On the other hand, other known isomers of HCH; namely, those that are alpha, beta, delta, and epsilon, were found to be too large. The gamma HCH isomer is commercially known as lindane and shows very strong excitation in the treated organism. Mullins further hypothesized that all of the HCH isomers could be pushed into the lattice-space, in an end-on orientation of the molecule, but then none fitted tightly enough to the walls of the adjacent axons. Mullins showed that DDT, if pushed into the lattice space in an end-on position, fitted rather tightly, and was therefore prepared to extend his steric concept to DDT activity. To augment his hypothesis, Mullins noted that 2-chloro-DDT cannot be correctly oriented; and thus, explains its activity.

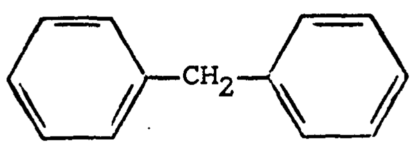
Although Mullins' hypothesis has never been disproved, it remains still entirely speculative and awaits confirming experimental evidence. It would appear that the key experimental results would be those equivocally showing lindane or another active agent actually binding or adhering to some vital surface of the axon membrane and thereby altering the axon's or its membrane's properties.

Mullins' steric hypothesis may be contrasted with the almost purely electronic explanation by F.A. Gunther and his co-workers. They examined the role of bonding energies, which they speculated were entirely of the van der Waals type. Gunther and his colleagues examined 30 DDT analogs, and treated the data by considering the substituted phenyls together as a "head" and the substituted alkane portion as the "tail." The DDT molecule may be diagrammatically represented as follows:

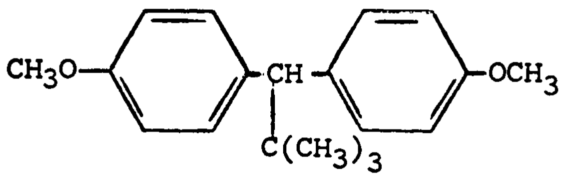


The various R's could be Cl, H, or $CH_3$, so that for any tail, for instance, one could have six different heads; namely, 1. Cl and Cl; 2. H and H; 3. $CH_3$ and $CH_3$; 4. Cl and H; 5. Cl and $CH_3$; and 6. H and $CH_3$. Of the many tails that could be made, they chose five; namely, 1. H, H, and H; 2. H, H, and $CH_3$; 3. H, Cl, and Cl; 4. H, H, and Cl; and 5. Cl, Cl, and Cl. They reported that if one end of DDT or one of its analog, a head or a tail, were held constant, then the toxicity for mosquitoes (Culex quinquefasciatus) increased linearly with each logarithmic increment of the total van der Waals forces of the chlorines plus the methyls plus the hydrogens. The slopes of the eleven graphs prepared in this way were remarkably constant in eight cases, varying only between 0.5 and 0.7. Unfortunately, neither correlation coefficients nor the graphs themselves were reported. However, if one were to assume that the graphical fit was good, then the implication is that by increasing nonspecific bonding forces one can improve affinity for a hypothetical target whose shape is complementary to DDT, and hence increase potency. Just why it is the logarithms of the van der Waals forces, rather than the force values themselves, is not clear. It may imply that it becomes progressively more difficult to improve the affinity, so that the logarithm of the van der Waals forces via successive additions of DDT or its analogs will achieve only linear toxicity increments. In other words, it is a simple case of diminishing marginal return for each successive input. The underlying assumption is that the fit to the target is comparable in all the compounds, and indeed chlorine and methyl groups have fairly similar van der Waals radii, 1.80 and 2.27 Å, respectively. Now hydrogen has a very different radius, 0.3Å, so one might imagine the H-substituted analogs to fit badly, and it is perhaps this that causes the slopes for an all-hydrogen head or all-hydrogen tail abnormal; i.e., 1.6 and 0.1, respectively. Yet in four out of the eight cases in which the slopes were so-called normal, there was at least one hydrogen in the head or tail of the whole group, and one would have thought that in these cases a different relationship would hold. The conclusions in Gunther's study was as follows: "These data are consistent and therefore in agreement with our postulate that these particular insecticides are reacting with a protein-like substance, presumably an enzyme." This conclusion may overstate the experimental evidences because there was really nothing in the paper which would indicate an enzymatic mechanism. Further, there was no indication in the experimental evidences whether the target is proteinaceous or not.

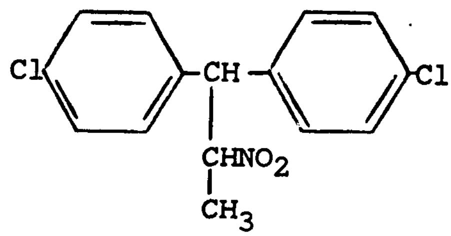
At this point of the narrative it may be appropriate to mention some of the very extensive structure-activity studies on DDT analogs, for they relate to the structural view of DDT action which is particularly appropriate to hypotheses that demand a close fit into a lattice or onto a membrane component. E.F. Rogers and his colleagues suggested in 1953 that the important property of the $CCl_3$ group was its bulk. If one considers the diphenylmethylene nucleus:



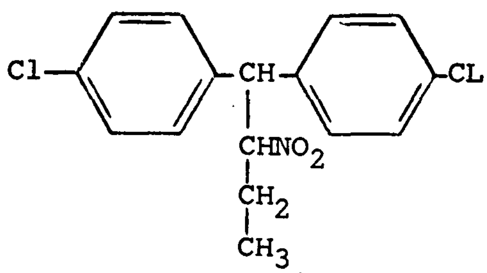
then, if substituents on the methylene group are small, the phenyl groups rotate freely around their bond to the methylene. But if a bulky substituent is inserted on the methyline, such a rotation is inhibited, especially if one imagines the bulky substituent "demanding" room to rotate freely, and the phenyls then take up the well-known "butterfly" configuration seen in DDT, in which the phenyls are as coplanar as they can be. Rogers concluded that coplanarity was necessary in the DDT molecule to have insecticidal properties. He apparently based the conclusion on the fact that bulky substituents other than $CCl_3$ were also effective insecticides. Thus, the unchlorinated compound dianisyl neopentane had good insecticidal activity. Rogers' hypothesis also helps to explain the toxic properties of Prolan [2-Nitro-1,1-bis(p-chlorophenyl)propane] and Bulan [1,1-Bis(p-chlorophenyl)-2-nitrobutane]. On the other hand, it turns out that if one replaces the $CCl_3$ group of DDT by $CH(NO_2)Cl$, or $C(CH_3)_2Cl$, or $C(NO_2)Cl_2$, these compounds have no insecticidal properties.



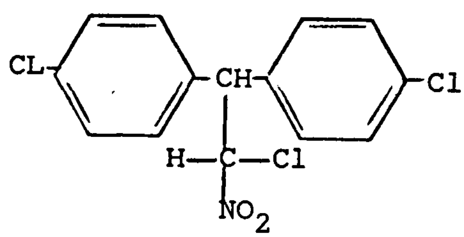
Dianisyl neopentane (active)



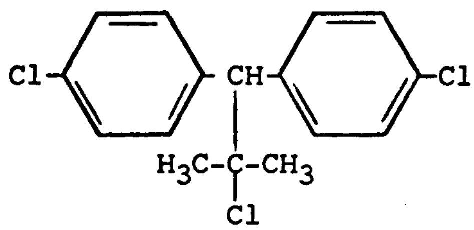
Prolan (active)



Bulan (active)



not active

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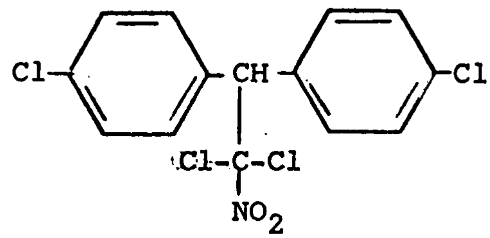
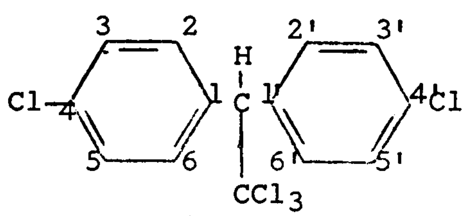
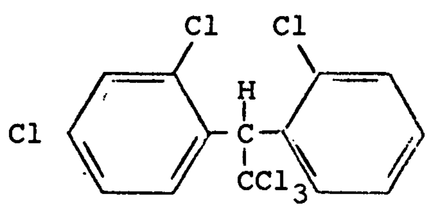



not active

Another hypothesis attempting to explain the toxic mechanism of DDT seems at first almost the opposite of Regers' "bulk" explanation. R. Riemschneider and H.D. Otto argued that some ability of the phenyls to rotate was a requirement for activity. However, in harmony with Rogers' view, these investigators considered that free rotation was required to permit the taking up of the "almost planar" configuration described above. This explains, these investigators reasoned, the o,o'-DDT isomer's lack of insecticidal properties because the two chlorines in the ortho positions in the phenyl groups restrict their rotation around the 1 carbon atom of the ethane molecule.

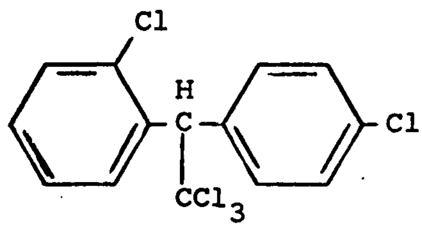



p,p'-DDT
(free rotation of phenyl groups; an active agent)




o,o'-DDT
(restricted rotation of phenyl groups; an inactive, agent)

Similarly, one could account for the properties of analogs with two methyl substituents per ring; when these were in the 2 and 4 positions, or the 2 and 5, rotation was impossible and the insecticidal activity lacking, but when they were in the 3 and 4 positions, rotation was possible and activity was found. The "partial rotation" hypothesis has one major flaw. The o, p'-DDT isomer does not have such rotability; yet it is a perfectly good insecticide.




o,p'-DDT
(restricted rotation of phenyl groups; an active agent)

The very extensive studies on structure-activity relationships in DDT analogs, mostly carried out before 1950, have been admirably reviewed by A.W.A. Brown in his book, Insect Control by Chemicals. Investigations centered on other compounds built on the 1,1-diphenylethane framework. More specifically, the points at which the DDT molecule were modified to produce new compounds, and the trends in synthesis during these early investigations of new analogs were as follows:

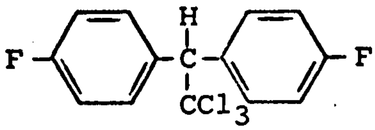
1. Alteration of the positions of the chlorine atoms on the benzene rings to produce structural isomers of DDT.

2. Replacement of the chlorine atoms with other halogens.
3. Substitution of other radicals on the benzene rings.
4. Subtraction, or addition, of chlorine atoms on the trichloroethane location in the molecule.

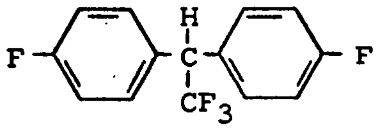
Compounds developed along these lines of synthesis are given in Tables 4 and 5, in which their relative insecticidal activity is indicated.

Table 4. Toxicity of DDT Analogs: Substitution of Other Halogens, and Structural Isomers

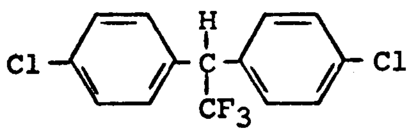
(The number indicates the relative degree of toxicity)



(4)



(2)



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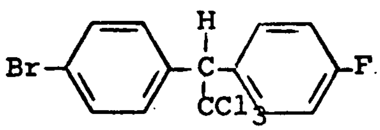

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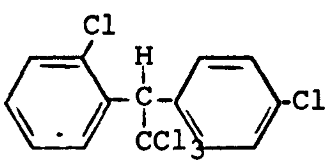

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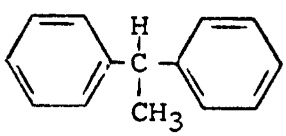

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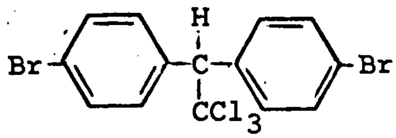

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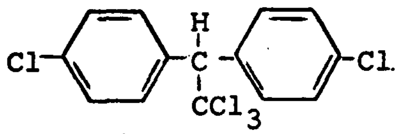

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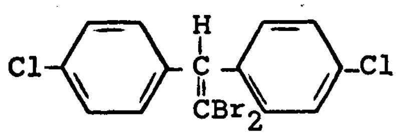

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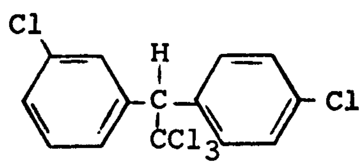

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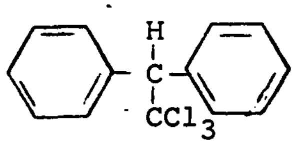

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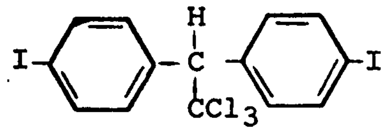

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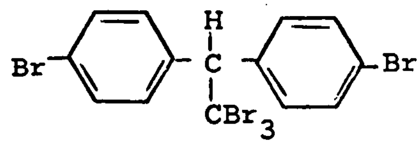

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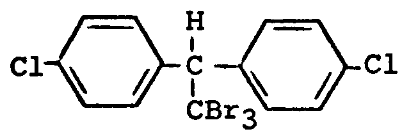

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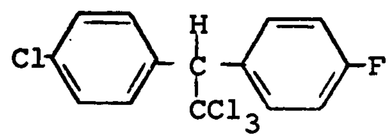

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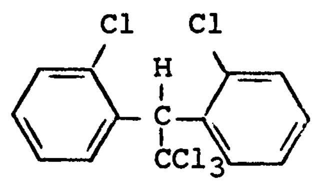

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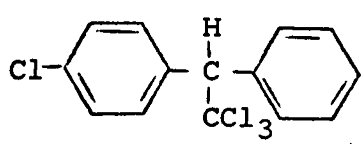

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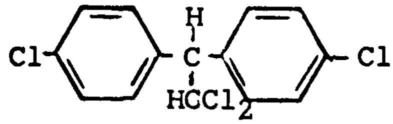
Table 5. Toxicity of DDT Analogs: The Effect of Removing Chlorine from the Ethane Nucleus and Substituting Other Groups on the Benzene Rings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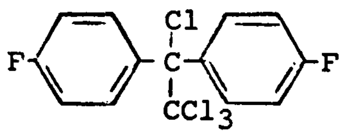
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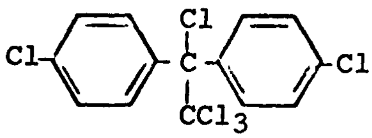
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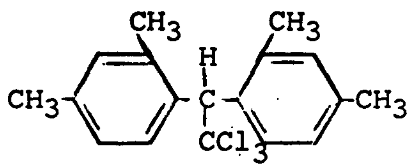
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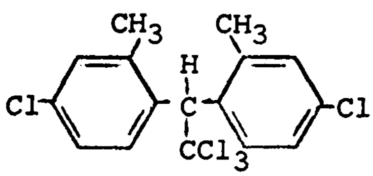
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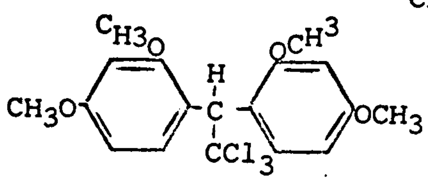
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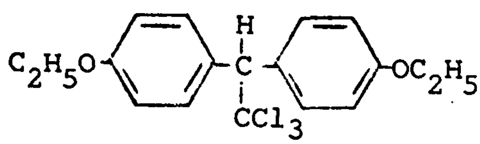
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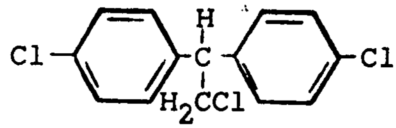
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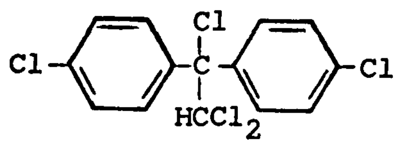
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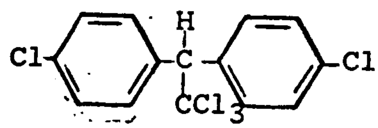
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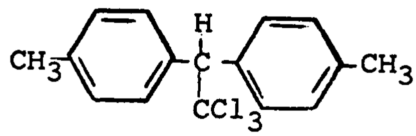
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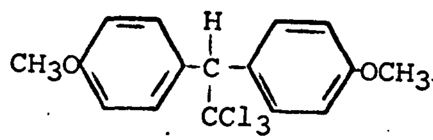
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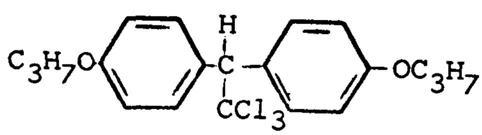
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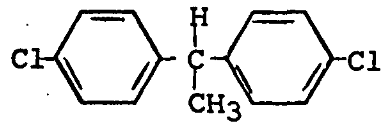
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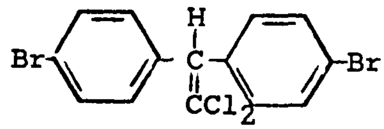
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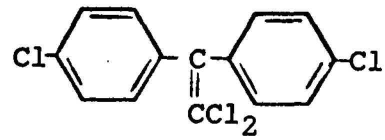
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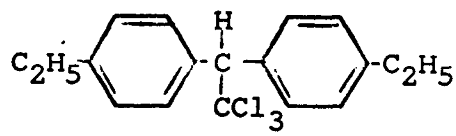
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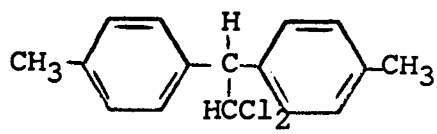
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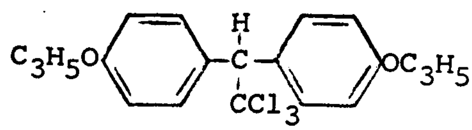
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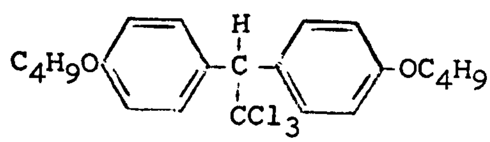
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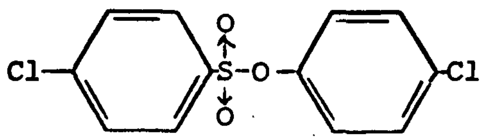
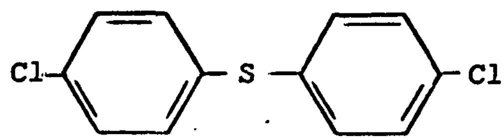
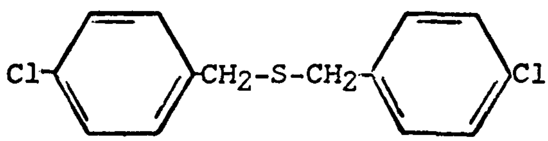
Additionally, it was observed by several early investigators that the most toxic of the diphenyl sulfo esters, sulfides, sulfoxides, and sulfones, diphenyl ethers, and phenyl benzyl ethers were those in which the benzene rings were halogenated in the p,p' position. These early investigations, although primarily concerned with stomach poisons for the clothes moth, led to the discovery of DDT, a contact poison rather than a stomach one. Chemical structures for some of the above diphenyl sulfur compounds are as follows:



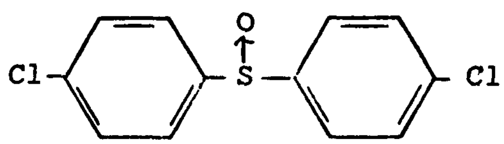
a diphenyl sulfo ester



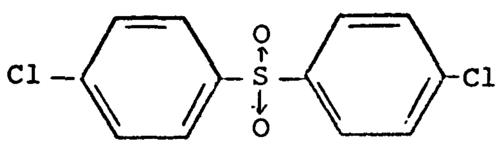
a diphenyl sulfide



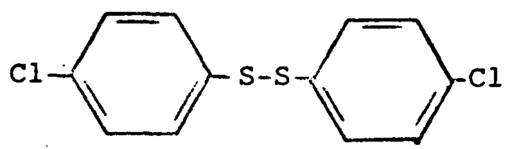
a dibenzyl sulfide



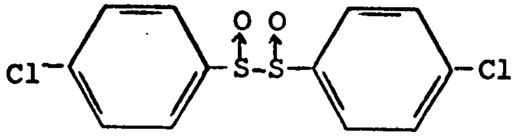
a diphenyl sulfoxide



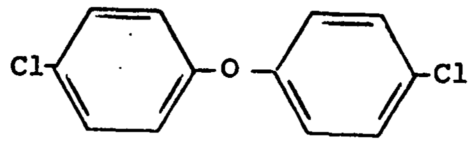
a diphenyl sulfone



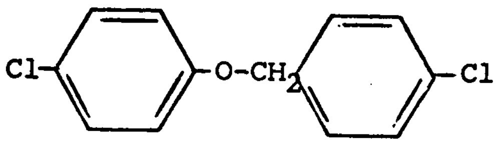
a diphenyl disulfide



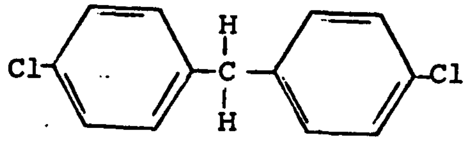
a diphenyl disulfoxide



a diphenyl ether

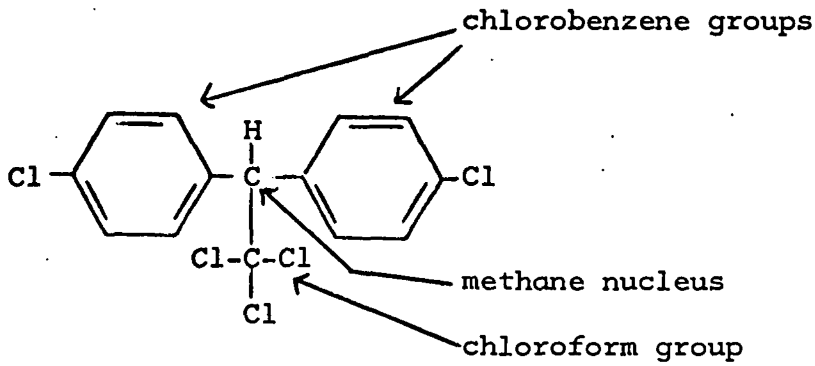


a phenyl benzyl ether



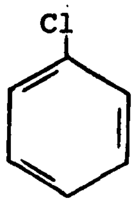
a diphenyl methane

Substitution of the sulfoxide group by a trichloroethyl group, i.e, [H-C-$CCl_3$], which is also strongly electronegative, confers lipoid solubility on the resulting molecule of DDT. Thus, the symmetrical apolar molecule of DDT is capable of showing toxic action by mere contact of DDT with the lipoid epicuticle of insects. An early hypothesis suggested that the high contact toxicity of DDT may be related to its molecular structure with a lipoid—soluble narcotic. This explanation has some merit on the grounds that the DDT molecule may be regarded as methane substituted with two chlorobenzene groups and a chloroform group.

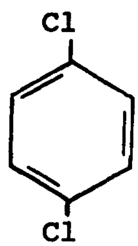



DDT

Chlorobenzene is toxic to insects, and addition of a second chlorine in the para position produces the well-known highly toxic fumigant, p-dichlorobenzene.

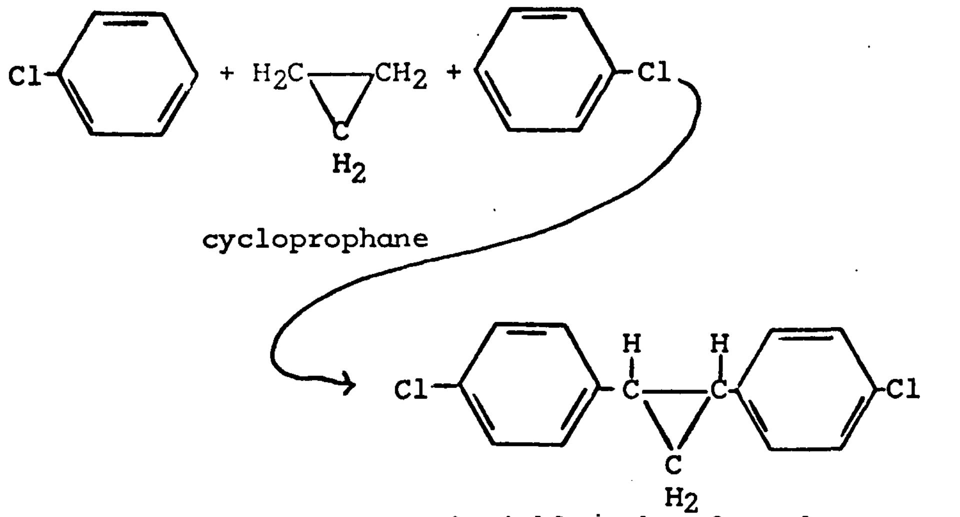


chlorobenzene



p-dichlorobenzene

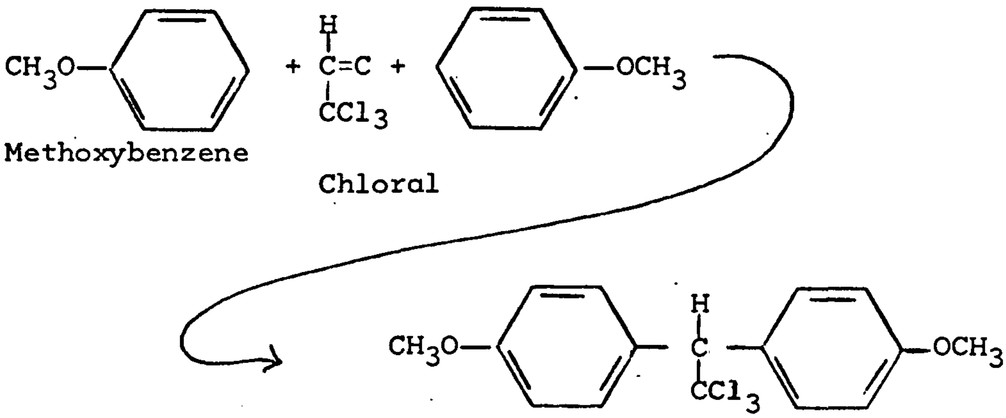
Chloroform is a strong narcotic and is soluble not only in the lipoids of nerve sheaths but also in the waxy epicuticle of insects. Combination of two moles of chlorobenzene with one mole of other narcotics; such as, bromoform $[HCBr_3]$, chloromethane $[H_3CCl]$, nitromethane $[H_3CNO_2]$, ethylene $[H_2C{=}CH_2]$, diethyl ether $[C_2H_5OC_2H_5]$, and divinyl ether $[H_2C{=}CHOCH{=}CH_2]$, also produced excellent contact insecticides, and condensation with the anaesthetic cyclopropane resulted in a contact insecticide of outstanding effectiveness.



cycloprophane

p,p'-Dichlorophenyl cyclopropane

On the other hand, it has been suggested that it is the chlorobenzene group which confers lipoid solubility and the remainder of the molecule is responsible for the toxicity. In methoxychlor, it is the methoxybenzene portion of the molecule which is lipoid soluble, whereas methoxybenzene is not toxic to insects.

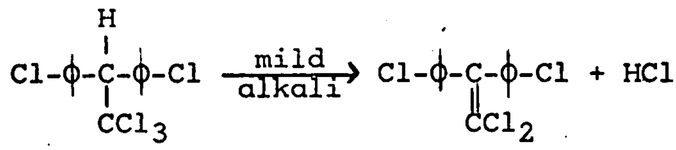


Methoxybenzene

Chloral

Methoxychlor

It was further suggested that the toxicity of DDT is related to the release of toxic HCl in the insect tissues, since the insecticide is highly susceptible to dehydrohalogenation by mild alkali. The reaction is as follows.



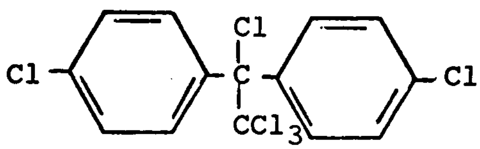

Careful examination was made by early investigators of the relation between dehydrohalogenation and toxicity in DDT and a large number of its analogs. Some general correlation was found with DDT analogs classified in three categories, that are:

1. Compounds split off HCl readily in the presence of alcoholic KOH. Some of the compounds in this category include DDT, DFDT (1,1,1-Trichloro-2,2-bis (p-fluorophenyl) ethane), and DDD (1,1-Dichloro-2,2-bis(p-chlorophenyl) ethane).

2. Compounds split off HCl slowly in the presence of alcoholic KOH. Some of the compounds in this category include methoxychlor (1,1,1-Trichloro-2,2-bis (p-methoxyphenyl) ethane), methyl-DDT (1,1,1-Trichloro-2,2-bis (p-methylphenyl) ethane), and methyl-DDD (1,1-Dichloro-2,2-bis(p-methylphenyl)ethane).

3. Compounds that split off HCl with comparative

resistance in the presence of alcoholic KOH. None of the compounds in this category show any more than the slightest toxicity. Dichlorodiphenyltetrachloroethane (1,1,1-Trichloro-2-chloro-2,2 bis(p-chlorophenyl) ethane),



is an example.

The susceptibility of the isomers of DDT to dehydrohalogenation follows their order of toxicity as follows: p,p'-DDt, o.99%; m,p'-DDT, 0.87%; o,p'-DDT, 0.10%; and o,o'-DDT, 0.0%. A similar correlation is evident in the halogenated analogs; i.e., fluoro chloro bromo iodo, in both the DDT and DDD series. All the dichloroethylene derivatives are less toxic than the parent trichloroethane analogs. This fact could be interpreted to indicate that they have no toxic HCl to liberate in the tissues by dehydrohalogenation of the ethane nucleus.

When the question of lipoid solubility is examined, it is found to bear no relationship to contact toxicity. Although DDT is appreciably liposoluble, most of its analogs, including the non-toxic ones, are considerably more so.

It may be concluded that although there is ageneral relationship of toxicity with: 1. the p-chlorophenyl group, 2. the lipoid-soluble or narcotic nucleus of trichloroethane, 3. solubility in lipoids, and 4. susceptibility to dehydrohalogenation, the following important exceptions can be found in each of the early hypotheses attempting to explain the toxic mechanism of DDT:

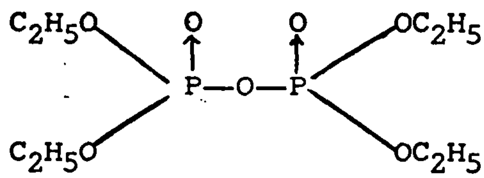
a. Methyl-DDT and methoxychlor are highly toxic.

b. There are analogs lacking the trichloroethane nucleus which are no less toxic than DDT and are more lipoid-soluble than DDT.

c. The relation of toxicity with lipoid solubility is erratic, and more inverse than direct.

d. Methoxychlor, methyl-DDT, and methyl-DDD are comparatively resistant to dehydrohalogenation.

Before ending the discussion on the mechanism of DDT action, two other recent hypotheses, the "toxin" and cholinesterase concepts, should be mentioned. The toxin hypothesis was introduced by J. Sternburg and C. W. Kearns in 1952. These workers observed that cockroaches which had been poisoned with DDT contained in their blood a factor which could kill flies and cause DDT-like effects; i.e., DDT "jitters", when applied to untreated cockroach nerve cords. This factor was not DDT itself; for, unlike DDT, it was not ether extractable. Since this observation, others have shown that a variety of stressful treatments cause cockroaches to produce such factors in their blood. For instance, pinning cockroaches down so that they struggle for days or putting them on a kind of treadmill that forces them to walk continuously for many hours, alters their blood composition so that transfer of it into untreated cockroaches causes paralysis or death. Other chemicals than DDT, specifically tetraethyl pyrophosphate (TEPP) and dieldrin, have also induced a blood-borne paralysis factor in cockroaches.



TEPP

tetraethyl pyrophosphate

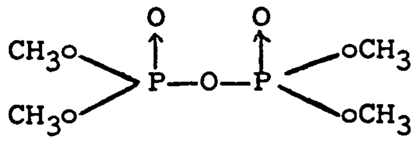
The observations suggest a refined version of an early view that death from DDT was "due to exhaustion," caused perhaps by excessive activity induced in sensory nerves. The refinement would consist in having a chemical factor, induced by DDT or other stressful treatments, as the immediate cause of death. It seemed that the story was complete when it was shown in 1958 and 1959 that certain heart cells (corpora cardiaca) of cockroaches have a sort of "neuroactive principle" which reduces the spontaneous activity of an isolated nerve cord, and this principle was depleted from these heart cells by stimulating the cockroaches electrically. It was also shown that both respiration rates and acetylcholine levels of cockroach nerve, and that cockroach strains tolerant of DDT were also tolerant of mechanical stress.

However, findings of later researches made this apparently attractive stress "toxin" hypothesis more complex. It turns out that there are three separate blood factors: (a) A heart accelerating factor released by mechanical stress in a particular type of heart cell (corpus cardiacum). The factor is also released in the nerve cord, brain, blood, and other organs. This factor has no effect on nervous action. (b) A neuroactive factor present only in the heart cells. The factor whose depletion by electrical stimulation is described above; and (c) the neuroactive factor which appears in the blood of DDT-poisoned cockroaches. Factors (b) and (c) have no effects on heart rate. These two factors are also readily separable chromatographically. However, factor (c) chromatographs the same way as the neuroactive factors found in the blood of mechanically stressed cockroaches, which contained no factor (b). In summary, it does seem that DDT and mechanical stress produce in blood, a neuroactive principle which plays an important role in causing deaths.

Later studies on this principle have yielded only moderate information concerning the DDT-induced factor, but have no led to an elucidation of the factor's chemical structure. What is known about the factor, other than its existence, is that it is dialyzable and it losses activity on standing in the presence of cockroach blood. The factor can be chromatographed on paper. Spot color tests on chromatograms suggested that the neuroactive factor (b) was an aromatic amine containing an ester group.

The "toxin" theory could, in principle, be compatible with the theories on interference with the nerve membrane. One might argue that DDT causes toxin production, which then causes membrane disruption. However, the toxin is detectable only after fairly prolonged DDT treatment; and furthermore, the underlying notion is that hyperexcitability in the sensory nervous system is the equivalent of mechanical or electrical excitation, and gives rise to toxin production. Therefore, the "toxin" hypothesis does not offer an explanation for the observed primary lesion in the nerve membrane after it is treated by DDT. It is the lesion which is presumed to be membrane destabilization.

A hypothesis which runs like an undercurrent through the literature on DDT is that the compound has an effect upon cholinesterase, an enzyme which catalyzes the hydrolysis of acetylcholine to acetic acid and choline. There were early reports that DDT was a cholinesterase inhibitor in vitro, but these findings have been either unconfirmed or specifically denied. J. M. Tobias and colleagues reported in 1946 that poisoning of the American cockroach or housefly by DDT led to a two-fold elevation in the acetylcholine level in the nervous system at the late stages of poisoning; cholinesterase was unaffected. Some controversey surrounded these experimental evidences; i.e. whether the compound found was acetylcholine or trichloracetic acid or something else. A thorough investigation by E. H. Colhoun in 1959 confirmed the delayed 27-fold rise in acetylcholine in the nerve cord of DDT-poisoned cockroaches. However, this occurred in 24 hours after treatment. The acetylcholine level remained at this high level for about another 36 hours. After that period, the level declined rapidly and dropped below the normal level during the last stages of poisoning when the cord becomes or is already necrotic. From these observations, Colhoun and colleagues suggested that acetylcholine synthesis took place at an elevated rate during the initial stages of poisoning, but in a form unavailable for hydrolysis by cholinesterase. He supports his suggestion by the fact that free acetylcholine was found in the nerve cord along with cholinesterase which, judged by subsequent homogenization and assay, was not inhibited. Colhoun and others concluded that the acetylcholine effect was only a secondary effect of DDT poisoning. However, in 1962, J. Sternburg and P. Hewitt pointed out that cholinesterases assays made subsequent to homogenization could give erroneous results, if the cholinesterase was inhibited reversibly in vivo. Such an inhibitor would be diluted away upon homogenization and thusly, they reasoned, inhibition of cholinesterase to hydrolyze acetylcholine would disappear. To make their point, they made ingenious use of an organophosphate; i.e., tetramethyl pyrophosphate (TMPP).




TMPP
Tetramethyl pyrophosphate

They selected the compound as an "irreversible" anti-cholinesterase, which hydrolyzes spontaneously and rapidly, so that it eliminates itself within a few hours.

When the control and DDT-treated groups of cockroaches were treated with TMPP, Sternburg and Hewitt observed that the resultant cholinesterase inhibition was far less in the DDT-treated insects. They reasoned that the cholinesterase of the DDT-treated cockroaches was protected from inhibition by TMPP. The protection increased as DDT poisoning deepened. No such protection was afforded when cockroaches were treated with lindane. It was also observed that when temperature was manipulated during TMPP induction, there was a reversal of DDT-poisoning symptoms; thus, temperature removes the protective effect. In other words, at lower temperatures DDT jitters in the treated insects were more severe than at higher temperatures. The temperature effect will be explored further in ensuing paragraphs of this discussion. One can argue that there may be other factors other then a protective inhibition factor in

DDT-treated insects against the anticholinesterase property of TMPP. The following alternative possibilities to explain the protective factor were experimentally disproved by Sternburg and Hewitt:

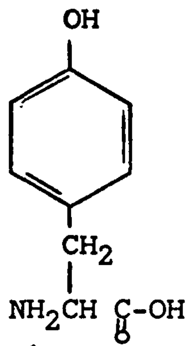
1. Poor circulation existed in the DDT-treated insects.
2. TMPP did not penetrate the DDT-treated insects' nerve cords.

At least two mechanisms for the protective effect can be speculated. 1) DDT, or a derivative of it, or a compound whose production or release is stimulated by it, might be a reversible inhibitor of cholinesterase, thus protecting it from phosphorylation. Putting this speculated mechanism more simply, there may be something in the DDT-treated nerve cord, and not present in the untreated ones, that protects the cholinesterase enzyme from being attacked by TMPP; namely, phosphorylation. It is unlikely that DDT itself affords this protection to cholinesterase. If it did, an effect would have been found at high concentrations in vitro. 2) The well-established increased levels of acetylcholine might be protective; i.e. a case of protection by substrate rather than protection by a reversible inhibitor. There is, however, evidence against acetylcholine protection, for J. B. Waller and S. E. Lewis have shown that lindane, aldrin, and pyrethrin cause similar increases in acetylcholine, but, as mentioned above, lindane does not protect the cholinesterase against phosphorylation action of TMPP.

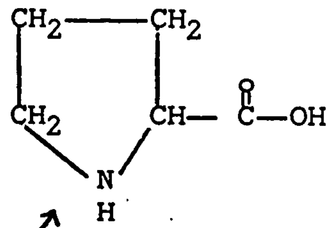
A determination of which of these alternatives is correct is of major importance: if DDT provokes reversible inhibition of cholinesterase, the increases in acetylcholine might be a result, and the mechanism could be important in explaining central effects of DDT. But if it is the excess acetylcholine that is protective, then the TMPP findings tell us nothing more than that acetylcholine is elevated, a fact well established by more direct evidence.

The temperature effect will now be briefly discussed in the following paragraphs. A well-documented fact is that DDT shows a negative temperature coefficient of activity, i.e., it is more active at low than at high temperatures. Insects treated with the appropriate dose can be cooled to 15°C and thrown into violent symptoms, then warmed to 35°C and appear entirely normal. This temperature effect is observed in various insects that have been treated with DDT. The effect is peculiar with DDT because it is the opposite to that of other types of insecticides; such as, organophosphates, pryethrins, and several other chlorinated hydrocarbons. This negative temperature effect of DDT seems to suggest that a chemical complex, formed by DDT and some other component in the nervous system, may be the basis of the toxic mechanism of DDT. A complex formation is suggested because virtually any type of complex formation indicate just such a negative temperature dependence, presumably because thermal agitation is disadvantageous for complexes. By contrast, chemical reactions usually show a positive temperature coefficient. In 1964, J. L. Eaton and J. Sternburg described an interesting analysis of DDT's temperature effect. They showed that the destabilization of sensory nerves by DDT gave a positive temperature coefficient, but that in the central nervous system showed a negative one. The overall response to DDT poisoning also showed a negative coefficient. These findings suggest that central nervous system phenomena are the more crucial in the DDT poisoning process. This position, however, conflicts sharply with the generally held view that sensory nerves are of primary importance.

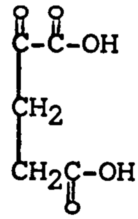
In summary, relatively coherent hypotheses of the mode of DDT's action have been sketched in this discussion. Many other bits and pieces of information exist pertaining to the subject matter, that seemingly do not fit into any of the hypotheses so far. For example, it has been experimentally demonstrated that DDT causes a sharp and substantial increase in oxygen consumption in all the insects studied so far. One explanation for this phenomenon is that increased uptake of oxygen results from excessive muscular activity, which in turn was caused by excessive nervous activity resulting from DDT. It has also been experimentally demonstrated that amino acid metabolism is disturbed by DDT. In 1963, J. J. Corrigan and C. W. Kearns showed that treatment of cockroaches with DDT sufficient to give symptoms at 15°C led to alteration in blood amino acid levels. For example, tyrosine, proline, and α-Ketoglutaric acid levels fell 71, 61, and 50 percents, respectively. On the other hand, phenylalanine level rose 131 percent.



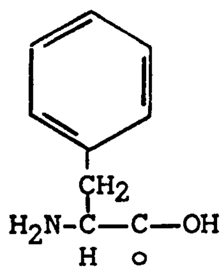
tyrosine
(α-amino-B-[p-hydroxyphenyl]propionic acid)



proline
(pyrrolidine-2-carboxylic acid)

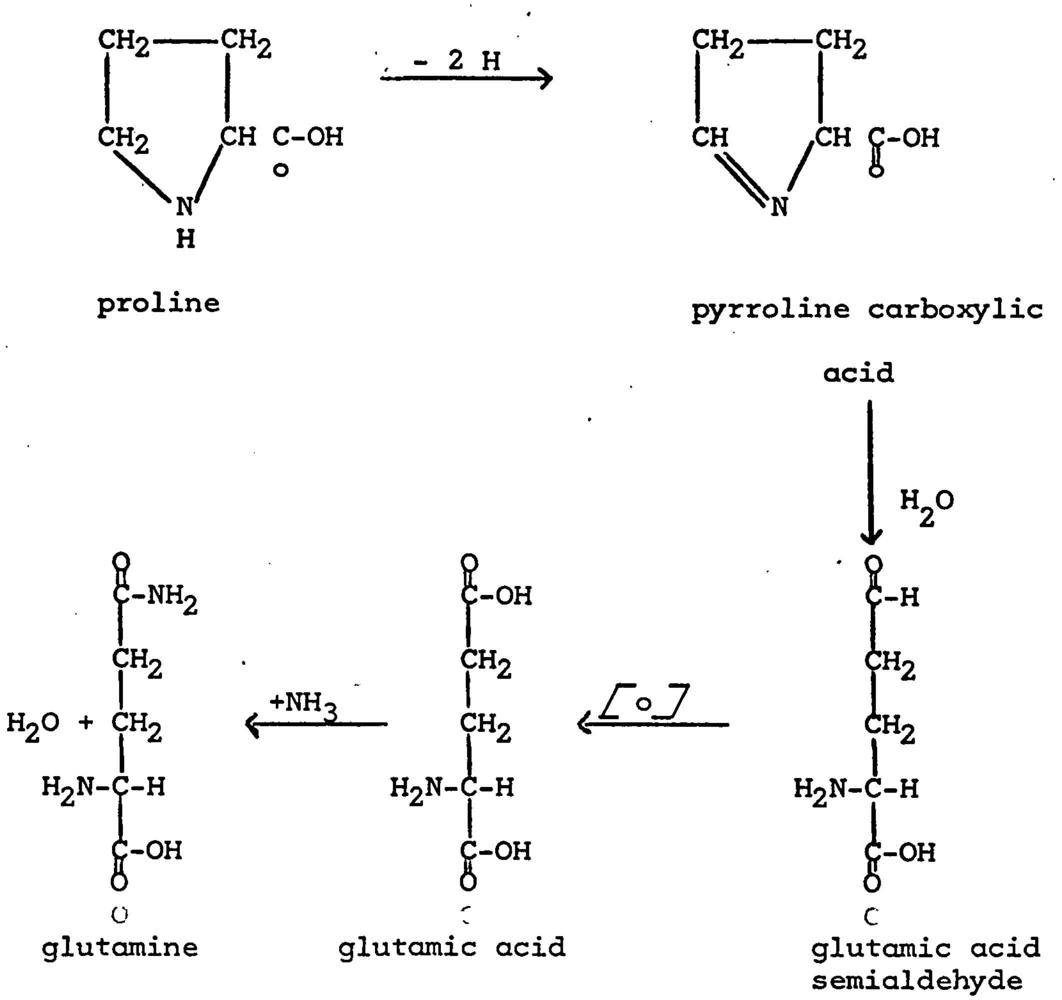


α-Ketoglutaric acid



phenylalanine (α-amino-B-phenylpropionic acid)

Corrigan and Kearns explored the proline effect further. When the temperature was raised to 34°C, the effect disappeared and the proline level returned to normal. When radioactively-tagged proline; namely; $C^{14}$-proline, was injected into cockroaches, it was observed that the DDT-treated insects respired two to three times more of the tagged proline as $C^{14}O_2$ than did the untreated ones. Tht $C^{14}$-proline was not only respired more, but three times more of it was converted to glutamine than was converted in controls. Corrigan and Kearns suggested that this extra proline ulitization was simply a reflection of a "demand for oxidizable carbon." The conversion of proline to glutamine probably follows the equations as given below:




The discussion above is an attempt to present briefly the most cogent hypotheses to explain DDT's toxic mechanism. The full and unequivocal elucidation of the insecticide's mode of action still remains to be discovered.

Toxicological Effects:

A. Fish and Other Aquatic Biota

The toxicity of DDT to fishes has been subjected to considerable study. Among the variables that have been cited are the type of water course and bottom, depth, vegetation, silt, turbidity, hardness, temperature, dissolved oxygen, organic content, species and age of fish, various commercial product formulations, volume and flow of water, and size and shape of receptacles. The effect of differing DDT formulations on rainbow trout have been cited as: 1. DDT in acetone solution, not lethal at 30 mg/l; 2. DDT in fuel oil, not lethal at 20 mg/l; 3. DDT in xylene, toxic at 5 mg/l; 4. DDT in emulsion, toxic at 3 mg/l; 5. DDT in kerosene, toxic at 0.3 mg/l.

Owing to these many variables, it is not surprising to find that there is a variation in concentrations of DDT lethal to fish as reported in the literature. The effects of various concentrations of DDT on different fishes are given in Table 6.

Table 6. Toxicity of DDT to Various Species of Fish

| Fish Species | Concentration (mg/l) | Results |
|---|---|---|
| Common sucker | 0.001 (alcohol suspension) | Killed |
| Speckled trout | 0.001 (alcohol suspension) | Killed |
| Common sucker | 0.005 | Killed |
| Speckled trout | 0.005 (alcohol suspension) | Killed |
| Creek chub | 0.01 (alcohol suspension) | Killed |
| Bluegill | 0.01 | Toxicity threshold |
| Dace | 0.01 | Killed |
| Gambusia | 0.01 | $LD_{50}$ |
| Guppies | 0.01 | Toxicity threshold |
| Bluegill fingerlings | 0.01 | Killed |
| Bass fingerlings | 0.01 | Killed |
| Bass fry | 0.025-0.04 | Killed |
| Rainbow trout | 0.0237-0.074 | 96-hour $TL_m$ |
| Bluegills | 0.04 | Killed |
| Salmon, young | 0.047 | 24-hour $TL_m$ |
| Bass fingerlings | 0.05 | Killed |
| Goldfish | 0.1 | Toxic limit |
| Goldfish | 0.1 | $LD_{50}$ |
| Bluegill adults | 0.1 | Killed |
| Bluegills | 0.14 (in fuel oil) | Killed |
| Darters | 0.14 (powder) | Killed |
| Sculpins | 0.14 (powder) | Killed |
| Trout | 0.14 (powder) | Killed |
| Bluegill fingerlings | 0.15 | Killed |
| Bluegills & Crappies | 0.18 | Killed |
| Goldfish | 0.20 | Killed |
| Fatheads | 0.40 | Killed |
| Golden shiners | 0.50 | Killed |
| Goldfish | 1.60 | Killed |

Table 7 gives the median lethal concentration ($LC_{50}$) of DDT for various species of fish.

Table 7. The $LC_{50}$ for Various Fish to DDT

| Fish Species | Exposure Time(hr) | $LC_{50}$ 1/ (mg/l) |
|---|---|---|
| Brook trout | 36 | 0.0323 |
| Land locked salmon | 36 | 0.08 |
| Mosquito fish | 36 | 0.32 |
| Largemouth bass | 96 | 0.002 |
| Brown trout | 96 | 0.002 |
| Coho salmon | 96 | 0.004 |
| Redear sunfish | 96 | 0.005 |
| Black bullhead | 96 | 0.005 |
| Rainbow trout | 96 | 0.007 |
| Bluegill | 96 | 0.008 |
| Yellow perch | 96 | 0.009 |
| Carp | 96 | 0.010 |
| Channel Catfish | 96 | 0.016 |
| Fathead minnow | 96 | 0.019 |
| Goldfish | 96 | 0.021 |
| Goldfish | 96 | 0.027 |

With increasing time and declining temperature the $LC_{50}$ to DDT for rainbow trout decreased from 0.012 mg/l to 0.0041. This fact indicates that DDT is negatively temperature dependent and positively time dependent. Data is given in Table 8.

Table 8. Effects of Time and Temperature on the Toxicity of DDT to Rainbow Trout averaging approximately one gram

| Temperature, °F | $LC_{50}$ (mg/l) 24 hrs. | 48 hrs. | 96hrs. |
|---|---|---|---|
| 45 | 7.5 | 4.7 | 4.1 |
| 55 | 8.2 | 5.2 | 5.0 |
| 65 | 12.0 | 7.3 | 6.0 |

1/ $LC_{50}$, median lethal dose, is the milligrams of toxicant per kilogram of body weight lethal to 50 percent of the test animals to which it is administered under the conditions of the experiment.

Resistance to DDT may be induced up to a certain concentration. For example, guppies which had been exposed to sublethal doses of DDT for 14 days and then placed in a toxic concentration of DDT (0.032 mg/l) demonstrated that they had increased their tolerance to the toxicant by this procedure.

Cutthroat trout were exposed in the laboratory for 30 minutes once a month for 1.5 years to the following quantities of DDT in water baths: 0.01 mg/l, 0.03 mg/l, 0.1 mg/l, 0.3 mg/l, and 1.0 mg/l. By the end of the experimental period from about 50 to 75 percent of the 636 fish in each group were dead at the three highest quantities of DDT. The number and volume of eggs produced by the trout were not reduced by these levels of DDT, but mortality among sac fry was higher at the 0.3 and 1.0 mg/l levels of DDT.

Mosquito fish collected from waters near cotton fields heavily treated with chlorinated insecticides exhibited significant levels of resistance to DDT, compared with fish from unexposed areas. A concentration of 0.05 mg/l of DDT caused only about a 20 percent mortality in the resistant fish, whereas this same concentration caused about a 90 percent mortality in the susceptible or unexposed fish. In another study about five percent of mosquito fish surviving after exposure to DDT at concentrations above the threshold toxicity aborted their young.

Some species of fish are extremely sensitive to DDT. For example, the extrapolated $LD_{50}$ dosage for young chinook and coho salmon was 0.0275 and 0.064 mg/kg per day, respectively. The chinook salmon appeared to be two to three times more sensitive to DDT than were coho salmon.

Atlantic croakers were fed 2.57 micrograms of DDT per gram weight of fish for 67 days. The accumulation of DDT resulted in mortality starting on the 14th day and continuing until all fish were dead by the 67th day. DDT is not only toxic to fish but may also alter the normal behavior of fish. For example, it was found that New Brunswick salmon from a DDT-sprayed region were unusually sensitive to low temperatures and selected water of higher temperature than usual. If this response occurred in nature, salmon might place their eggs in regions where the young fry could not survive. It was also found that mosquito fish exposed to low levels of DDT in the range of 0.1 to 20 parts per billion (ppb) for 24 hours tended to prefer waters with a higher level of salinity than unexposed fish. The amount of DDT taken up by pinfish reached a maximum level about two weeks after exposure to dosages of 0.1 ppb and 1.0 ppb. At this time, the pinfish had residues ranging from 3.8 ppm to 11.5 ppm. DDT residues in coho salmon eggs from Lake Michigan measured during 1968 ranged from 1.1 to 2.8 ppm. Mortalities in the salmon fry after hatching ranged from 15 to 73 percent. The higher residues of DDT in the eggs of these salmon were generally correlated with higher mortalities in the fry.

In Idaho and Wyoming, treatment of forests with DDT at 1, 2.5, 5, and 7.5 pounds per acre influnced some fish populations. At the one pound per acre dosage in Idaho, some cottids (Cottus beldingii), mountain suckers, and black bullheads were killed by the DDT, but speckled dace, redside shiners (Richardsonius balteatus hydrophlox), rainbow trout, eastern brook trout, and cutthroat trout apparently were not affected. A few cutthroat trout were killed by the 2.5 pound per acre application of DDT. The most striking influence of DDT was on the diet of fish. Before treatment, there was no crayfish in the diet, but immediately after the treatment the percentage increased to 99 percent, as in the case of the brook trout sampled. No measure of the long term effects of the change in food organisms was made in the investigation.

A spray calculated to give a DDT content of 0.09 ppm in water was used to treat a stream. Eight miles downstream from the treated area hundreds of fish were reported dying, and the concentration of DDT at a point ten miles downstream was 0.017 ppm. In 1955 when the fish hatchery on Lake George lost all of nearly 350,000 eggs removed from lake trout, DDT was suspected as the cause. For several years, about 10,000 pounds of DDT had been distributed yearly for control of gypsy moth and biting flies in the watershed associated with Lake George. Careful studies revealed that DDT stopped reproduction of lake trout in Lake George and several other heavily contaminated lakes in the adjacent Adirondack region. Although the trout eggs contained from 3 to 355 ppm of DDT, little or no mortality occurred in the egg stage. The fry, however, were highly sensitive to these dosages and were killed at the time of final absorption of the yolk sac, just when they were ready to feed. For example, at levels of DDT in eggs that would produce 3 ppm in fry, few fry survived; and at 5 ppm DDT, none survived.

The spraying of New Brunswick forests with DDT between 1953 and 1958 was reported to be responsible for the severe reduction in salmon fishing success in the province. The severest reduction occurred in 1959 and 1962. In a succeeding investigation, the mortality of young Atlantic salmon and eastern brook trout was observed in cages and free-living streams in forested areas of New Brunswick sprayed with DDT for spruce budworm control. There were no short-term effects on salmon parr with DDT at one-quarter pound per acre, but many yearlings were killed. Two applications of one-quarter pound per acre ten days apart were as harmful as a single application of one-half pound per acre. DDT at one-half pound per acre caused a loss between 50 to 98 percent of underyearling and parr salmon. In a similar investigation, applications of DDT to control nuisance insects appeared to be associated with the decline of the salmon fishery at Sebago Lake, Maine. Average DDT residues in salmon collected in 1962, 1963, and 1964 were, respectively, 1.1, 3.2, and 1.8 ppm by total weight. Salmon in the three year age group had 1.2 ppm; in the four year age group, 8.0 ppm; and in the five year age group, 8.8 ppm of DDT.

It appears that there is a link between the feeding habits of Atlantic Salmon and DDT. For example, the application of DDT at one-half pound per acre to a forest watershed of the Northwest Miramichi River, New Brunswick, changed the kinds of food found in stomachs of young Atlantic salmon. Salmon under one year typically consume immature aquatic insects, whereas salmon over one year consume all sizes of aquatic insects. After the DDT application, the surviving young salmon fed on worms, snails, and fish which previously had been unimportant in their diet. With the resurgence of aquatic insects, the young salmon went back to its pre-spray feeding habits.

Observations in the field confirm laboratory findings that DDT is highly toxic to some fish and especially to fry. For example, one investigation reported that when levels of DDT and its metabolites were above 400 parts per billion in the eggs of hatchery trout, the "mortality in the resulting fry ranged from 30 to 90 percent in the 60-day period following the swim-up stage." In a similar investigation, it was reported that DDT residues in coho salmon eggs from Lake Michigan measured during 1968 ranged from 1.1 to 2.8 ppm. Mortalities in the fry after hatching ranged from 15 to 73 percent. The higher residues of DDT in the eggs of these salmon were generally correlated with higher mortalities in the fry. DDT's high toxicity to salmon fry, parr, and underyearlings would seemingly account for the relatively low commercial salmon catches during and in immediately succeeding years in which salmon spawning waters were heavily contaminated with DDT.

Fish also accumulates DDT. In one investigation reporting the effects of an application of one pound per acre of DDT over a 72,000-acre area in the Yellowstone River drainage in 1957 for spruce budworm control, DDT was found up to 0.03 ppm in the water. Samples of mountain whitefish, rainbow trout, and brown trout contained DDT up to 14.00 ppm and DDE (Dichlorodiphenyldichloroethylene), a degradation product of DDT, up to 6.53 ppm. The author further reported that "DDT was found in trout 85 miles below the spray area, and fish taken more than two years after the spraying still contained DDT." In another report, DDT was applied at 0.2 pound per acre to a tidal marsh in Florida. Total kills of caged striped mullet, sheepshead, longnose killifish, rainwater killifish, and tidewater silverside occurred in 1 to 24 days. Fish accumulated up to 90 ppm of DDT within five weeks after treatment.

In addition to fish, DDT is toxic to amphibians, reptiles, arthropods, aunelids, and other aquatic insects. One investigation reported the impact of an application of one pound per acre of DDT for the control of tent catapillars in Hubbard County, Minnesota. Before spraying, 111 small frogs (Rana sylvatica) were counted around two pools. The frogs seemed well a day after spraying, but the water had oil film and was covered with dead poisoned caterpillars. Two and a half days later, 35 dead frogs were found, and after a few more days, no living ones remained. All but two of the 34 frog stomachs contained tent caterpillars, among other insects. Whether frogs were killed directly or indirectly by eating poisoned insects, the local population was drastically reduced. Later investigations showed that DDT is toxic toward frogs, especially the young. The 24-hour median lethal concentration of DDT for Fowler's toad tadpoles and chorus frog tadpoles was, respectively, 2.4 and 1.4 ppm.

Investigations showed that DDT is harmful for the p propagation of molluscs and that they accumulate the insecticide. For example, an investigation reported that seawater with a DDT level of 0.1 ppm halted the growth of eastern oysters, and dosages as low as 0.0001 ppm significantly reduced oyster growth. Eastern oysters containing about 151 ppm of DDT requires approximately three months in clean water to lose 95 percent of their load of DDT. Their growth returned to normal after only ten days of flushing in clean water. Several other mollusc species lost about 75 percent of accumulated DDT after 15 days of flushing in clean water. Table 9 gives the accumulation and retention of DDT by molluscs.

Table 9. Accumulation and Retention of DDT by Molluscs Exposed for Seven Days to 1.0 μg/l in Flowing Seawater and Then Placed in Clean Water

| Mollusc | Residue After 7 Days Exposure | After 15 Days Exposure | After 30 Days Exposure |
|---|---|---|---|
| Hooked mussel | 24 | --- | --- |
| Eastern oyster | 26 | 2.5 | 1.0 |
| Pacific oyster | 20 | 16.0 | --- |
| European oyster | 15 | 8.0 | 4.0 |
| Crested oyster | 23 | 5.0 | --- |
| Northern quahog | 6 | 0.5 | --- |

Fiddler crabs fed natural organic plant detritus for 11 days in estuaries containing DDT exhibited grossly modified behavior. Within five days on the DDT containing detritus, the crabs became uncoordinated. When threatened, they did not scurry away, but moved a short distance, lost coordination and equilibrium and rolled over.

DDT caused reductions in numbers of natural insect predators and parasites as well as the target insect species. There is also ample field study evidence to show increases of other pests that are DDT resistant once their natural predators are reduced. Some of the more salient evidences are given in ensuing paragraphs.

DDT was applied in apple orchards for the control of apple pests to eliminate populations of certain highly susceptible, predaceous ladybird beetles. As these beetles were the principal controlling agent for a red-mite pest, the mite population subsequently reached outbreak levels, causing severe damage to the apple trees. This particular mite is not susceptible to DDT and was therefore hardly influenced by the chemical which killed the beetle. Outbreaks of the red-handed leaf roller occurred in apple orchards after the use of DDT because the leaf roller's parasites and predators were more susceptible than the leaf roller. DDT applications sharply reduced the parasitism of the apple mealy bug, as DDT is highly toxic to its parasite. In another field study, it was found that DDT caused a reduction in numbers of natural predators, followed by an increase in numbers of European red mites and clover mites. The study showed the relationship between the concentration of DDT used and the time applied and the frequency and magnitude of population outbreaks attained by the mites. The study also showed the factors involved in reestablishing predator populations and the time required for reattainment of equilibrium of low populations of predators and mites. A similar field study concerned an attempt to eliminate caterpillars, in particular Pieris rapae, on cole crops with DDT. The study reported that the survival of the pest was better than expected because the insecticide killed many of the caterpillars' natural enemies. The study indicated that it was impossible to predict the changes in species populations with the application of any one insecticide to a biotic community. However, one common trend was the reduction or elimination of natural enemies, frequently leading to outbreaks in the numbers of herbivores or pest species on the cole crop under study.

Aquatic insects are particularly susceptible to DDT. An investigator studied the number and kind of aquatic insects present in the forest-covered tributaries of the Miramichi River of northern New Brunswick after aerial treatment with 0.5 pound per acre of DDT. In the streams affected by DDT fewer insect species emerged, and those species most severly reduced were the larger ones, such as caddice flies. The treated streams generally had larger numbers of individuals, but the weight of insect life was in some cases reduced by half. Furthermore, the insect fauna of the treated streams was deficient in the species of insects on which salmon mainly feed. This aspect was mentioned in earlier paragraphs of this discussion. From two to three years were necessary for the insect fauna to recover qualitatively for most groups; however, for some recovery required four years. In a similar field study, DDT was applied at 1.0 pound per acre for control of the spruce budworm to the Swan Creek drainage area in Montana. Although the spraying aircraft did not treat within one-quarter of a mile of the stream, 0.01 ppm of DDT was measured in the water one-half hour after spraying. Three hours after the application samples of insects contained up to 11 ppm of the insecticide. Extreme mortalities occurred in mayfly nymphs, caddice fly larvae, and stonefly larvae by one hour after treatment. These mortalities are not surprising in view of the massive dose and the susceptibility of these aquatic insects. Table 10 gives the median lethal concentration of DDT for some aquatic fauna.

Table 10. The Median Lethal Concentration for Various Arthropods to DDT

| Arthropod Species | Exposure Time(hr) | $LC_{50}$ (ppm) |
|---|---|---|
| Amphipod (Gammarus lacustris) | 24 | 0.0047 |
| Amphipod (Gammarus lacustris) | 48 | 0.0021 |
| Grass shrimp (Palaemonetes vulgaris) | 24 | 0.012 |
| Hermit crab (Pagurus longicarpus) | 24 | 0.007 |
| Sand shrimp (Crangon septemspinosa) | 24 | 0.003 |
| Stonefly (Pteronarcella badia) | 24 | 0.012 |
| Stonefly (Claassenia sabulosa) | 24 | 0.016 |
| Stonefly (Pteronarcys Californica) | 24 | 0.041 |
| Stonefly (Pteronarcys Californica) | 48 | 0.019* |
| Stonefly (Pteronarcys Californica) | 48 | 0.019* |
| Waterflea (Daphnia pulex) | 48 | 0.00036 |
| Waterflea (Daphnia pulex) | 48 | 0.0004 |

* Different investigations

In another investigation, DDT was applied at 0.5 pound per acre for control of the elm spanworm. In the one drainage area where precautions were not taken to avoid the stream, a 90 percent kill of mayfly and stonefly nymphs occurred. A similar incident occurred in Pennsylvania when 0.25 pound per acre of DDT was applied directly to a stream. Approximately 90 percent of the total stream insect population was exterminated and about 35 percent of the species eliminated. Some species did not repopulate the stream for two years or more. After the treatment of 72,000 acres of the Yellowstone River system with DDT at one pound per acre, stream-bottom invertebrates were significantly reduced in number. Total numbers of invertebrates had recovered within a year, but the species composition was still altered. Both the folded-wing insects (Plecoptera) and the very short-lived insects (Ephemeroptera) were significantly reduced, but both the hairy-winged insects (Trichoptera) and two-winged insects (Diptera) occurred at higher numbers at the end of one year.

DDT is also toxic toward earthworms and insect larvae. In one field investigation, DDT applied at 25 pounds per acre reduced earthworm activity by 80 percent. Earthworm populations were found to reflect the dosage of DDT in soil. In soils containing 26.6 ppm, 4.1 ppm, and 3.6 ppm, the earthworms in these soils averaged about 14 ppm, 7 ppm, and 3 ppm, respectively.

DDT application at 0.1 ppm for control of black fly larva in Bobby's Brook, Labrador, resulted in several faunal changes. Caddice fly larval populations were reduced to zero or near zero at all stations receiving the treatment, and the same was true for stonefly and mayfly larvae. The DDT also caused mortalities in eastern brook trout by contamination of the fish foods above maximum tolerance levels.

Insects exposed to DDT have apparently become resistant themselves or produced progenies that are resistant to the insecticide. For example, honeybees at Riverside, California, were found to be six times more resistant to DDT than honeybees from unexposed areas. One investigator reported that approximately 225 species of insects and mites have evolved resistance to DDT. Increasing number of insects have also evolved resistance to, in addition to DDT, cyclodiene (aldrin, dieldrin, etc.) and organophosphorus (parathion, malathion, etc.) types of insecticides. The 225 species were broken down as follows: 121 crop pests, 97 man and animal pests, 6 stored-product pests, and 1 forest pest. These results provide an idea of the amount of DDT and other insecticides in the environment and intensity of selective pressure.

Resistance to DDT may be induced by the accumulation of the insecticide, especially if the dosages were sub-lethal and small. Biological concentration of DDT is especially prevalent in molluscs and crustacea. Eastern oysters placed in flowing seawater containing 1 part per billion (ppb) of DDT for 40 days concentrated DDT some 70,000 times the level in the water. Oysters exposed for ten days to a mixture of eight pesticides in the water, ranging from 0.001 to 0.05 ppm, increased the pesticide concentrations in their bodies; DDT, for example, was concentrated 15,000 times.

Fish and birds also concentrate DDT and other pesticides. Croakers, a saltwater fish, concentrated DDT 20,000 times the level in water containing 0.001 ppm of the insecticide. After two weeks of exposure to 0.001 ppm of DDT in water, ten fish concentrated the level of DDT in their bodies 12,000 times the level of the water. When ten fish were exposed to a lower concentration of DDT at 0.0001 ppm, they were found to be able to concentrate the level in their own bodies 40,000 times that of the water. DDT residues were found to reach a level of more than 13 pounds per acre in a Long Island saltmarsh. In a sampling of a freshwater estuary and a saltmarsh and the organisms therein, DDD in the water was estimated at 0.05 ppb. The organisms consisting mostly plankton had about 40 ppb of DDT, a biological concentration factor of 800. The highest concentrations were detected in the scavenging and carnivorous fish and birds; the birds were reported to have 10 to 100 times more than the fish species.

In Lake Michigan sediments averaged 0.014 ppm of DDT, DDE, and TDE. From the same habitat the amphipod averaged 0.41 ppm for DDT and its related metabolites, or about 30 times the level found in the mud; various fish removed from the lake had varying amounts of the insecticide's residues. Alewives had 3.35 ppm; chub, 4.52 ppm; and whitefish, 5.60 ppm. The breast muscle of gulls averaged 90.5 ppm of DDT, approximately 27 times that found in alewives. And body fat of the gulls averaged 2,441 ppm of DDT!

The chemical attributes of DDT make it susceptible to biological concentration in algal living systems. For example, four species of algae concentrated DDT about 220-fold when exposed to a concentration of DDT a 1 ppm in water for seven days. Daphnia, a zooplanktonic organism, concentrated DDT 100,000-fold during a 14-day exposure to water containing 0.5 ppb of DDT. A fathead minnow concentrated DDT further in its tissues on being fed Daphnia containing DDT. Depending upon the DDT concentration in the medium and in the food, fish and crustacea have been observed to concentrate DDT from 50 to over 200 times that of its medium's level. For example, it was observed in ponds containing 0.02 ppm of DDT in water, rainbow trout, black bullhead, and crayfish concentrated DDT to the levels of 4.15 ppm or 208 folds, 3.11 ppm or 156 folds, and 1.47 ppm or 74 folds, respectively.

In addition to aquatic biota and waterfowl, soil organisms; such as earthworms and soil insects, also accumulate DDT. Animals and birds feeding on these insects further contentrate DDT in their bodies. It was observed in one investigation that in some DDT-sprayed elm environments, pesticide residues accumulated from 9.9 ppm in the soil to 141 ppm in earthworms; and, in turn, to 444 ppm in brains of adult robins. In another area where elm trees had been sprayed with DDT for control of Dutch elm disease, the soils had a residue to 19 ppm of DDT and earthworms from the same soil contained 157 ppm.

DDT is an extremely persistent insecticide. Soil residues in a Maine forest treated with DDT at one pound per acre showed little decrease during the nine years after application. It was estimated that DDT residues may persist over 30 years. Supporting this estimation are results from another investigation, which reported that the percentage of DDT applied at a rate 100 ppm to sandy loam soil remaining after 17 years was still 39 percent.

Toxicological Effects:

B. Higher Animals

The median lethal concentration ($LC_{50}$) of DDT and DDE for various birds is given in Table 11.

Table 11. The $LC_{50}$ of DDT and DDE for Various Birds*

| | DDT(ppm) | DDE(ppm) |
|---|---|---|
| Mallards | 850 to 1,200 | 3,300 to 3,600 |
| Pheasants | 300 to 700 | 750 to 950 |
| Bobwhites | 600 to 1,000 | 750 to 950 |
| Coturnix | 400 to 600 | 1,200 to 1,400 |

* Insecticides in diets of two-week-old birds when fed treated feed for five days followed by untreated feed for three days

Pheasants were maintained on diets containing different dosages of DDT for an experimental period of 90 days. In the test, three out of ten females on 600 ppm of DDT died; all the four males on 400 ppm of DDT died, whereas the 20 females on this dosage all survived; and one out of the ten females on 200 ppm of DDT dies.

DDT has been reported not only to be toxic to birds but also to cause significant changes in the physiology of some species of birds. DDT fed daily to pheasant hens at 10, 100, and 500 ppm DDT in their food produced a normal number of eggs which were fertile and hatched satisfactorily. However, chick mortalities were reported to be highest from parents who received 500 ppm of DDT. In another investigation, it was found that only 44 percent of the eggs laid by herring gulls on the Lake Michigan side of the Door County peninsula were observed to hatch, as compared with a 90 percent level of hatching found in the same species in Denmark. This reduction was reportedly due to the higher level of DDT and its metabolites found in the Michigan gull eggs.

Bird population reduction since the advent of DDT and other insecticides is also attributed to eggshell thinning. This is especially true of raptorial and upland game birds. Bald eagles fed controlled dosages of DDT in the laboratory proved to be susceptible to DDT. The median lethal dose for eagles is estimated to be 80 ppm of DDT. The investigators pointed out that this level produced chronic poisoning. The dosage also suppressed reproduction and thinned eggshells.

To see if there was a correlation between the thinning of eggshells and DDT and/or its metabolites, historical comparisons of eggshell weights and thicknesses were made. In one historical survey, 614 peregrine falcon eggshells were both weighed and measured for thickness. Eggs collected in California from 1947 to 1952 had a significant decrease in both thickness and weight of eggshells, compared with those collected in the same area from 1895 to 1939. A regression analysis was run between shell thickness and total DDE residues in herring-gull eggs collected in Maine, Michigan, Minnesota, Rhode Island, and Wisconsin. A high correlation was found between the level of DDE residue and the thickness of the eggshell; i.e., the more DDE residue, the thinner the eggshell. The weights of raptorial birds' eggshells in museums and private collections were also measured to determine if there had been a change in the weights of these eggshells from the "pre-DDT" period of 1886 to 1939 to the "post-DDT" period of 1947 to 1962. In Brevard County, Florida, bald eagle eggshells from the pre-DDT period weighed 12.15 $\pm$ 0.127 grams. Eggshells from the post-DDT period weighed 9.96 $\pm$ 0.280 grams. Hence, there was an 18-percent decrease in the weight of the eggshells. Reports were also received that the bald eagle population was declining in this area. Similar results were reported from Osceola County, Florida. From 1901 to 1944, the mean weight for bald eagle eggshells was 12.32 $\pm$ 0.240 grams. From 1959 to 1962 the mean weight declined to 9.88 $\pm$ 0.140 grams, a decline of 20 percent. Bald eagle populations were reported to have declined also during the post-DDT years. The mean weight of 117 osprey eggshells taken between 1880 through 1938 was 7.08$\pm$ 0.069 grams, whereas the mean weight of six osprey eggshells collected in 1957 was 5.30$\pm$ 0.446 grams, a decline of 25 percent. It was also reported that osprey population in the area also decreased during the post-DDT years.

A Canadian investigation report indicated a 11 percent drop in the thickness of prairie falcon eggshells during the post-DDT years compared to those collected prior to the advent of DDT and other halogenated hydrocarbon insecticides. The report indicated a high correlation between the decline in the thickness of these eggshells and DDE content in the eggs. Associated with this decline was a 34-percent decrease in the occupancy of territories unknown to falcons during the previous ten years. The relatively high content of DDT and its metabolites found in raptorial birds and in their eggs are not surprising because these birds are at the end of a food chain.

Laboratory experiments confirmed the eggshell thinning phenomenon attributed to DDT or its metabolites. In one experiment, American sparrow hawks were fed for two years a diet containing DDE, a dosage equivalent to residue levels commonly found in the foods of raptorial birds in the field. The investigators reported that there was no difference in eggshell thickness between the treated and non-treated birds during the first year. However, they noted an average ten percent decline in the thickness of eggshells from treated hawks in the succeeding year. In another experiment, DDT and dieldrin were fed in combination to the hawks. Eggshells from the treated hawks were, on the average, also thinner than the controls. The investigators also noted that there were also fewer eggs laid by the treated hawks.

The decrease in fertility was also observed in DDT-dosed coturnix and ringdoves. One investigation team reported that coturnix fed p,p'-DDT in their feed at dosages of 2.5, 10, and 25 ppm for 26 weeks produced overall 18 to 21 percent fewer eggs than did the control group. Downward production trends continued for both the 10 ppm and 25 ppm dosages with time. Eggs produced by the three DDT-treated groups of birds had, respectively 6.0, 6.4, and 7.3 percent thinner eggshells than the untreated ones. It was also observed that hatching success declined significantly with time in all groups except those fed 2.5 ppm doses. In another investigation, mature coturnix were fed 0, 100, 200, and 400 ppm of DDT in their feed for 60 days. No effect on mortaliby, egg hatchability, or fertility was observed inthe 100 and 200 ppm group. However, the 400 ppm group suffered a 50 percent mortality within 30 days after treatment began. This group also showed a marked decline in fertility. There was some decrease in hatchability of eggs from this group. Young chicks hatched from this group exhibited incoordination of muscular action and spasms.

Another team of investigators reported that coturnix, fed relatively high dosages of DDT, produced eggs with significantly less calcium. The same team also reported that the shell-forming glands of treated birds had 16 to 19 percent lower carbonic anhydrase activity than the untreated ones. The eggshells from the treated group were ten percent thinner than the control group. Physiological changes brought on by DDT and its metabolites were noted in experiments with ringdoves. In one experiment, a group of ringdoves fed 10 ppm of DDT in the feed showed a significant decrease of estradiol in the blood. Moreover, this decrease occurred early in the breeding cycle.

Consequently, egg-laying was delayed from a normal 16.5 $\pm$ 1.6 days to 21.2 $\pm$ 5.5 days. The eggshells from the treated group had approximately ten percent less weight than those from untreated birds.

A high correlation was found also between the amount of DDE, a major matabolite of DDT, in eggs and eggshell thickness of pelicans. An important consequence of eggshell thinning is the premature cracking of the eggs. On Anacapa Island off the coast of California, egg breakage resulted in the complete reproductive failure of the brown pelican on the island during 1969. Shells of a few intact eggs measured shortly after egg-laying averaged only 0.38 millimeters (mm) compared to the average normal thickness of 0.57 mm, a decline of 34 percent. Residues of DDT and its metabolites were about 1,200 ppm, of which 85 percent was DDE. Residues in the fat of adult birds ranged between 738 and 2,603 ppm. The investigators concluded that "these findings, along with existing experimental evidence, clearly implicate DDE as a cause of eggshell thinning, reproductive failure, and population decline in brown pelicans." These findings were confirmed by similar field studies done by other investigators. For example, when weights and thicknesses of brown pelican's eggshells were compared between those collected prior to 1947 and in 1969, both measurements suffered decreases in the 1969 eggshells. The investigator further reported that the 16.2 percent decrease in eggshells of South Carolina pelican eggs was about the same for the eggshells of raptorial birds found in the area. Other investigators found that 15 pelican eggs taken in Texas and Florida after 1949 were on the average 20 percent below normal weight. Shell thickness was found to have decreased between 15 and 27 percent. One investigator advanced an explanation for the eggshell thinness phenomenon, that it appears to be due to changes in the storage and mobilization of calcium after ingestion, rather than action at the initial step of this process.

DDT's toxic effects toward birds and other non-target species have been a subject of many field investigations. Some of the more conclusive findings are given below.

After the application of DDT at two pounds per acre every year for four years, populations of American red-starts, parula warblers, and red-eyed vireos in forested areas declined 44, 40, and 28 percent, respectively, over the four-year period compared with the non-sprayed area. Elm trees in a 430-acre area were sprayed with six-percent DDT for control of disease. The soil in the area contained up to 18 ppm and the earthworms contained from 53 to 204 ppm. The median DDT residue found in 21 dead robins was 3 mg. If this was taken as the lethal dose for robins, it would take less than 100 worms from the area for a robin to accumulate the lethal dose of 3 mg/kg of its body weight. In Hanover, New Hampshire elms were treated with 1.9 pounds per acre of DDT. The spraying resulted in the deaths of 151 birds compared to only ten in an untreated area in nearby Norwich, Vermont. Moreover, the robin immigrant population in Hanover by June 1, 1963, had declined to 70 percent below the original May 1 population level. While at Norwich, there was no net change. Other birds affected included the myrtle warbler and the tree swallow. DDT's disastrous effects on birds were also recorded in Michigan and Wisconsin. The insecticide was applied to elms on the Michigan State University campus for the control of Dutch elm disease. The application nearly killed all the robins as well as many other birds on the campus. Three habitats in Wisconsin received DDT for control of Dutch elm disease, and three areas were unsprayed. In the three DDT-treated areas, bird population averaged 31, 68, and 90 percent below those of the unsprayed areas. Robin populations in the sprayed areas were 69,70, and 98 percent below those of the unsprayed areas. Treatment of two areas in Wisconsin with DDT to control Dutch elm disease with about two pounds of the insecticide per tree resulted in a robin mortality ranging from 86 to 88 percent.

The breeding success of New Brunswick woodcocks was closely related to the amount of DDT used; i.e., an inverse relationship. From 1961 to 1963, the level of residues of DDT in spring woodcock arrivals in New Brunswick increased significantly from an average of 2.0 to 5.4 ppm DDT.

A survey reported that mortality among herring gulls found on the edge of Lake Michigan was attributed to DDT present in the area. Reproduction in these herring gulls appeared to be reduced by the presence of DDT. A sample of nine eggs which appeared to be alive contained dosages of 202 $\pm$ 34 ppm of DDE. The ten dead eggs sampled had a higher concentration of 919 $\pm$ 117 ppm of DDE. From 30 to 35 percent of the eggs in 115 nests were dead, and this was felt to be an exceptional egg mortality. An investigation of a rice-growing region in California where DDT-treated seed was used for pest control, pheasants were found to have concentrations of DDT averaging 740 ppm in their fat. The survival rate of young pheasants was lower than normal, prompting a restriction against planting DDT-treated seed. In an investigation of the effect of temperature and DDT spraying on the ruffed grouse population, a team of investigators reported an apparent interaction between these two factors. May and June temperatures were related to the time of nest initiation, to egg loss, and to other mortalities. A synergistic effect between DDT at levels of 0.25 and 0.5 pound per acre and temperature was apparent in the loss of partially developed eggs. The DDT treatment was also correlated with a loss of immatures and changes in fall age ratios.

DDT is acutely toxic to mammals on oral administration. The oral toxicity averages about a tenth of the intravenous route of administration. Toxicity varies in the same species according to absorption. In general, solutions of the toxicant are more toxic than the powder. Reported median lethal doses ($LD_{50}$) for the same species as well as for different species of mammals vary. Presumably these dosage variations are due to various factors such as temperature, prior exposure to the toxicant, purity of DDT, intrinsic factors in the individual test mammal, and other conditions peculiar to the individual laboratory experiment or field investigation. Some of the reported median lethal doses via ingestion for various mammals are: rat, 420 to 800 mg/kg; mouse, 200 mg/kg; rabbit, 250 to 400 mg/kg; dog, 60 to 75 mg/kg; guinea pig, 400 mg/kg.

The symptoms of DDT poisoning in mammals start with twitching of the eyelids and general hyperexcitability. The twitching progresses to severe generalized tremors of long duration. This is then followed by alternate, irregular contractions and relaxations of muscles characterizing a clonic spasm. The animal then undergoes continuous muscular tension or contraction characterizing a tonus convulsion. Depression, paralysis, and death follow in rapid succession. Animals that survive several weeks show extensive damage in the liver, kidneys, and spleen. There are generally no significant lesions in the central nervous system. Cumulative toxicity from repeated exposure to subtoxic doses has been accused of producing digestive derangements and various obsecure disturbances in man, but the connection appears very dubious due to lack of experimental verification. There are no definite data on continued DDT administration in man. It was estimated that two to five milligrams per kilogram of body weight would probably cause mild illness.

It has been experimentally shown that prolonged administration of the insecticide in mammals does not increase their susceptibility to the acute effects, but rather decreases the initial symptoms. For example, continued feeding of dogs with 100 mg/kg daily results in contractions of skeletal muscle. When the dose is increased to 150 and 250 mg/kg of body weight, severe but reversible neurological disturbances appear. These disturbances become increasingly irreversible with larger and larger doses. The neurological changes observed in the test dog resembled those symptoms associated with the removal of certain portions of the brain; i.e., cerebellum and cerebral cortex. The cerebellum of the test animal showed considerable damage due to DDT poisoning, especially at high dosage levels. Slow poisoning of rats with DDT begins with loss of appetite. Also during the early stages of poisoning certain changes occur in the fatty tissues of the rat's liver. The significance of these changes to the physiology of the test animal is a moot point. Moreover, these changes are reversible and apparently only occur in rodents, especially in male rats. Female rats, on the other hand, are more susceptible to general toxicity. When rats and mice are kept on a low fat diet, the toxic effects seem to decrease.

After administration of a large single dose, DDT is found widely distributed in the tissues, but especially in the fat. With continued ingestion, but without signs of toxicity, the amount stored in the body may exceed several times the amount that would be fatal with a single intravenous injection.

Through slow accumulation the amount of DDT on its metabolites in the fatty tissues may be up to ten times that found in the food. Storage in the fat of rats occurs even with low insecticide dietary levels and the concentration of DDT in these tissues increases indefinitely with the duration of ingestion, until the level reaches an equilibrium plateau. This equilibrium point or plateau is characteristic of the dosage. Smaller amounts of the insecticide or its metabolites are found in the lymph nodes, adrenals, heart, and thymus. Trace amounts are found in the testes, liver, kidney, spleen, central nervous system, and lungs.

When the administration of the toxicant is discontinued, to percent of the stored DDT is still present after a month and 25 percent after three months. Up to 70 percent of the toxicant as bis(p-chlorophenyl)acetic acid is eliminated in the urine of rabbits. The remainder is excreted very slowly. DDT is also eliminated via milk, so that this may produce typical toxic symptoms in the sucklings of poisoned animals. For example, milk cows fed on sprayed alfalfa hay contained about 3.5 mg/l. The butterfat may contain as much as 65 mg. Upon withdrawal of DDT contaminated feed, the toxicant level in the body burden rapidly falls off within ten days. However, small amounts still persist after six months of withdrawal.

The total number of reported poisonings in man by DDT is very small relative to the extent of its use; and clinical poisoning attributed to DDT is generally complicated by the possible toxic effects of solvents and sometimes by other insecticides in mixtures. No instance of chronic poisoning in man has been confirmed. Experiments on volunteers showed no effects after a single oral dose of 0.5 gram, and only minor DDT symptoms after 1.5 gram. Fatal poisoning in a child indicates that 150 mg/kg of body weight is lethal. If this dose is taken, the calculated lethal dose for an average adult weighing about 70 kg (approximately 150 pounds) would be ten grams. This dosage is similar to the median lethal dose for many mammals.

Clinical symptoms of acute DDT poisoning in man may start in half an hour with large doses; i.e., 300 to 500 mg via ingestion. Patients with mild cases have nausea, vomiting, anxiety, a burning sensation in the lips and face. In more severe cases, the patient may have, in addition, tremors, convulsions, stiffness and pain in the jaws, and soreness in the throat for several days. No instance of uncomplicated fatal poisoning by DDT is on record. However, a man who had swallowed about 120 c.c. of a commercial insecticide with five percent of DDT reported as the principle toxic ingredient developed blood poisoning, suppression of urinating functions, and involuntary spastic contractions of the fingers and wrists. The patient died in a deep coma in six days after ingestion. Autopsy revealed severe degeneration in the patient's renal tubes and liver cells. Although it is not certain that DDT was alone responsible, the symptoms and lesions are similar to those observed in animals.

# DDT

## General References

Books:

1. Brown, A.W.A., "Insect Control by Chemicals." John Wiley & Sons, Inc., New York, N.Y. 1951

2. Deichmann, W.B. and Gerade, H.W., "Toxicology of Drugs and Chemicals." Academic Press, Inc., New York, N.Y. 1969

3. Gudzinowicz, B.J., "Gas Chromatographic Analysis of Drugs and Pesticides." Marcel Dekker, Inc., New York, N.Y. 1967

4. McKee, J.E. and Wolf, H.W., "Water Quality Criteria." Second Edition. The Resources Agency of California, State Water Quality Control Board, Sacramento, Calif. 1963

5. Melnikov, N.N., "Chemistry of Pesticides." Springer-Verlag, New York, N.Y. 1971

6. Metcalf, R.L., "Organic Insecticides." John Wiley & Sons, Inc., New York, N.Y. 1955

7. Muller, P. (Editor), "DDT: The Insecticide Dichlorodiphenyltrichloroethane and its Significance." Birkhäuser, A.G., Basel, Switzerland 1959

8. O'Brien, R.D., "Insecticides: Action and Metabolism" Academic Press, Inc., New York, N.Y. 1967

9. Pimentel, D., "Ecological Effects of Pesticides on non-Target Species." Executive Office of the President, Office of Science and Technology, Washington, D.C. 1971

10. Sollmann, T., "A Manual of Pharmacology and its Applications to Therapeutics and Toxicology." Eighth Edition. W.B. Saunders Company, Philadelphia, Pa.

11. Tucker, R.K. and Crabtree, D.G., "Handbook of Toxicity of Pesticides to Wildlife." Resource publication No. 84. U.S. Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Washington, D.C.

12. von Oettingen, W.F., "The Halogenated Aliphatic, Olefinic, Cyclic, Aromatic, and Aliphatic-Aromatic Hydrocarbons including the Halogenated Insecticides, Their Toxicity and Potential Dangers." Public Health Service Publication No. 414. U.S. Department of Health, Education and Welfare, Washington, D.C. 1955

13. West, T.F., and Campbell, G.A., "DDT and Newer Persistent Insecticides." Chemical Publication Company, New York, N.Y. 1952

14. Wilber, C.G., "The Biological Aspects of Water Pollution." Charles C. Thomas, Publisher, Springfield, Illinois 1969

15. Zweig, Gunter, "Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives." Academic Press, New York, N.Y. 1964

## General References

**Articles:**

1. Abedi, Z.H., Duffy, J.R., and Brown, A.W.A., J. Econ. Entomol., Vol. 56, p.511 (1963).

2. Abelson, P.H., Ann. New York Acad. Sci., Vol. 69, p.276 (1957).

3. Acree, F., Beroza, M., and Bowman, M.C., J. Agr. Food Chem., Vol. 11, p.278 (1963).

4. Adams, L., J. Wildl. Manag., Vol. 13, p.245 (1949).

5. Adams, L. et al., J. Wildl. Manag., Vol. 13, p.245 (1949).

6. Adlung, K.G., Naturwissenschaften, Vol. 44, p.471 (1957).

7. Agosin, M. et al., J. Econ. Entomol., Vol. 54, p.340 (1961).

8. Allison, D. et al., Science, Vol. 142, p.958 (1963).

9. Anderson, B.G., Science Vol. 102, p.539 (1945).

10. Anderson, D.W. et al., Can Field Nat., Vol. 83, p.91 (1969).

11. Anderson, D.W. and Hickey, J.J., The Wilson Bull., Vol. 82, p.14 (1970).

12. Anderson, J.M., and Peterson, M.R., Science, Vol. 164, p.440 (1969).

13. Anderson, R.B., and Everhart, W.H., Trans. Am. Fish. Soc., Vol. 95, p.160 (1966).

14. Anon. Bio. Bull., Vol. 2, p.1., Dept. of Lands and Forests, Ontario, Canada (1949).

15. Atallah, Y.H., and Newsom, L.D., J. Econ. Entomol., Vol. 59, p.1181 (1966).

16. Atkins, E.J., and Anderson, L.D., J. Econ. Entomol., Vol. 55, p.791 (1962).

17. Azevedo, J.A., Hunt, E.G., and Woods, L.A., Calif. Fish and Game Vol. 51, p.276 (1965).

18. Baker, R.A., J. Water Pollution Control Fed., Vol. 33, p.1009 (1961).

19. Barker, P.S., and Morrison, F.O., Can J. Zool., Vol. 43, p.324 (1964).

20. Barker, R.J., J. Wildl. Manag., Vol. 22, p.269 (1958).

21. Beament, J.W.L., Insect. Physiol., Vol. 2, p.199 (1958).

22. Beard. R., Entomol. Exp. Appl., Vol. 8, p.193 (1965).

23. Berger, R.S., and Young, R.G., J. Econ. Entomol., Vol. 55, p.533 (1962).

24. Bitman, J. et al., Nature, Vol. 224, p.44 (1969).

25. Bitman, J., Cecil, H.C., Fries, G.F., Science, Vol. 168, p.594 (1970).

26. Bishop, E.L., Pub. Health Rep. No. 62, Dept. of Health, Education, and Welfare, Washington, D.C. (1947).

27. Bishopp, F.C., Jour. A.W.W.A., Vol. 39, p.704 (1947).

28. Bishopp, F.C., Jour. A.W.W.A., Vol. 40, p.264 (1948).

29. Blus, L.J., Bio-Science, Vol. 20, p.867 (1970).

30. Bogan, R.H., Okey, R.W., and Vargas, D.J., Research, Vol. 14, p.269 (1961).

31. Boswell, V.R. et al., U.S. Dept. Agr. Tech. Bull. 1121, Dept. of the Agriculture, Washington, D.C. (1955).

32. Bowman, M.C. et al., J. Econ. Entomol., Vol. 52, p.1038 (1959).

33. Bowman, M.C., Acree, F., and Corbett, M.K., J. Agr. Chem., Vol. 8, p.406 (1960).

34. Boyd, C.E., Progr. Fish-Cult., Vol. 26, p.138 (1964).

35. Boykins, E.A., Bio Science, Vol. 17, p.37 (1967).

36. Bridges, W.R., and Andrews, A.K., Spec. Sci. Report: Fisheries No. 391, Fish and Wildlife Service, Dept. of the Interior, Washington, D.C. (1961).

37. Brown, A.W.A., Can Med. Assoc. J., Vol. 100, p.216 (1969).

38. Buhler, D.R., Rasmussen, M.E., and Shanks, W.E., Toxicol. Appl. Pharm., Vol. 14, p.535 (1969).

39. Bull, D.L., and Adkisson, P.L., J. Econ. Entomol., Vol. 56, p.641 (1963).

40. Burden, E.H.W.J., Nature (London), Vol. 178, p.546 (1956).

41. Burdick, G.E. et al., Trans. Am. Fish. Soc., Vol. 93, p.127 (1964).

42. Cannon, M.S., and Holcomb, L.C., Ohio J. Sci., Vol. 68, p.19 (1968).

43. Carollo, J.A., Jour. A.W.W.A., Vol. 37, p.1310 (1945).

44. Christie, A.E., Water Sewage Works, Vol. 116, p.172 (1969).

45. Cline, R.E., and Pearce, G.W., Biochemistry, Vol. 2, p.657 (1963).

46. Colhoun, E.H., J. Agr. Food Chem., Vol. 8, p.252 (1960).

47. Cope, O.B., Trans. Am. Fish. Soc., Vol. 90, p.239 (1961).

48. Cope, O.B., J. Appl. Ecol., Vol. 3 (Supplement on pesticides in the Environment and their effects on Wildlife), p.33 (1966).

49. Cope, O.B., Trans. Sec. Sem. on Bio. Problems in Water Pollution Robt. A. Taft San. Eng. Center, Pub. Health Serv., Washington, D.C. (1959).

50. Corrigan, J.J., and Kearns, C.W., J. Insect Physiol., Vol. 9, p.1 (1963).

51. Cottam, C., and Higgins, E., J. Econ. Entomol., Vol. 39, p.44 (1946).

52. Couch, L.K., Trans. N. Amer. Wildl. Conf., Vol. 11, p.323 (1946).

53. Croker, R.A., and Wilson, A.J., Trans. Am. Fish. Soc., Vol. 94, p.152 (1965).

54. Crouter, R.A., and Vernon, E.H., Can. Fish. Cult., Vol. 24, p.51 (1959).

55. Cuerrier, J.P., Keith, J.A., and Stone, E., Natur. Can., Vol. 94, p.315 (1967).

56. Dale, W.E. et al., Science, Vol. 142, p.1474 (1963).

57. Datta, P.R. et al., Science, Vol. 145, p.1052 (1964).

58. Davey, K.G., J. Insect Physiol., Vol. 9, p.375 (1963).

59. DeBach, P., Calif. Citrograph, Vol. 32, p.406 (1947).

60. Dempster, J.P., J. Appl. Ecol., Vol. 5, p.451 (1968).

61. Dempster, J.P., J. Appl. Ecol., Vol. 5, p.463 (1968).

62. Dempster, J.P., Entomol. Exp. Appl., Vol. 11, p.51 (1968).

63. DeWitt, J.B., J. Agr. and Food Chem., Vol. 3, p.672 (1955).

64 DeWitt, J.B., J. Agr. and Food Chem., Vol. 4, p.863 (1956).

65. Diamond, J.B. et al., Canad. Entomol., Vol. 102, p.1122 (1970).

66. Doane, C.C., J. Econ. Entomol., Vol. 55, p.416 (1962).

67. DuBois, K. P., Postgrad. Med. Vol. 24, p.278 (1958).

68. Dunachie, J.F. and Fletcher, W.W., Ann. Appl. Biol., Vol. 64, p.409 (1969).

69. Eaton, J.L., and Sternburg, J., J. Insect Physiol., Vol. 10, p.471 (1964).

70. Edwards, C.A., Dennis, E.B., and Empson, D.W., Ann. Appl. Biol., Vol. 60, p.11 (1967).

71. Eide, P.M. et al., J. Econ. Entomol., Vol. 38, p.492 (1945).

72. Elson, P.F., and Kerswill, C.J., Trans. 20th N. Amer. Wildl. Conf. (1955).

73. Elson, P.F., and Kerswill, C.J., Atlantic Salmon J., Vol. 3, p.20 (1964).

74. Elson, P.F., J. Fish. Res. Board Can., Vol. 24, p.731 (1967).

75. Everhart, W.H., and Hassler, W.W., Trans. Am. Fish. Soc., Vol. 75, p.59 (1948).

76. Fairchild, C.G., and Barreda, E.A., J. Econ. Entomol., Vol. 38, p.694 (1945).

77. Ferguson, D.E. et al., J. Mississippi Acad. Sci., Vol. 11, p.239 (1965).

78. Frey, P.J., Spec. Sci. Report: Fisheries No. 391, Fish and Wildlife Service, Dept. of the Interior, Washington, D.C. (1961).

79. Fyfe, R.W. et al., Can. Field Nat., Vol. 83, p.191 (1969).

80. Gabrilevskaja, L., Water Pollution Abs., Vol. 28, p.2359 (1955).

81. Gaines, T.B., Toxic. and Appl. Pharma., Vol. 2, p.1 (1960).

82. George, J.L. et al., J. Wildl. Manag., Vol. 21, p.42 (1957).

83. Gilbert, F.F., J. Wildl. Manag., Vol. 33, p.933 (1969).

84. Gill, J.A., Verts, B.J., and Christensen, A.G., J. Wildl. Manag., Vol. 34, p.223 (1970).

85. Ginsburg, J.M., J. Econ. Entomol., Vol. 38, p.274 (1945).

86. Ginsburg, J.M., J. Econ. Entomol., Vol. 40, p.275 (1947).

87. Gjuilian, C.M. et al., J. Econ. Entomol., Vol. 42, p.100 (1949).

88. Gorham, J.R., "Aquatic Insects and DDT Forest Spraying in Maine". Maine Forest Service and the Conservation Foundation, Augusta, Maine, (1961).

89. Gotaas, H.B., Sewage and Industrial Wastes, Vol. 26, p.325 (1954).

90. Graham, R.J., and Scott, D.O., "Effects of an Aerial Application of DDT on Fish and Aquatic Insects in Montana". Final Report: Montana State Fish and Game Department and U.S. Forest Service (1959).

91. Gunther, F.A. et al., Arch. Biochem., Vol. 50, p.504 (1954).

92. Gunther, F.A. et al., J. Agr. Food Chem., Vol. 4, p.338 (1956).

93. Gunther, F.A. et al., J. Econ. Entomol., Vol. 51, p.385 (1958).

94. Hanson, W.R., J. Wildl. Manag., Vol. 16, p.299 (1952).

95. Harvey, J.M., Can. J. Zool., Vol. 45, p.629 (1967).

96. Hatch, R.W., Prog. Fish Cult., Vol. 19, p.89 (1957).

97. Hatch, R.W., Progr. Fish-Cult., Vol. 19, p.89 (1957).

98. Hatfield, C.T., Canad. Fish. Cult., Vol. 40, p.61 (1969).

99. Hawkins, W.B. and Sternburg, J., J. Econ. Entomol., Vol. 57, p.241 (1964).

100. Hayes, W.J., Jr., Proc. Conf. on Physio. Aspects of Water Quality Pub. Health Serv., Washington, D.C., (1960).

101. Hayes, W.J., Durham, W.F., and Cueto, C., Jr., J. Am. Med. Assoc., Vol. 162, p.9 (1956).

102. Hayes, W.J., Ann. Rev. Pharmacol., Vol. 5, p.27 (1965).

103. Hayne, D.W., Final Rep. to Bur. Sport Fish. and Wildlife, Dept. of the Interior, Washington, D.C. (1970).

104. Heath, R.G. et al., Patuxent Wildlife Research Center Bur. Sport Fish. and Wildlife, Washington, D.C. (1970).

105. Heath, R.G., Spann, J.W., and Kreitzer, J.F., Nature, Vol. 224, p.47 (1969).

106. Helle, W., Advan. Acarol., Vol. 2, p.71 (1965).

107. Henderson, C., Pickering, G.H., Tarzwell, C.M., Tech. Rep. W60-3, Biol. Probl. Water Pollu., Trans. 1959 Seminar, Robt. A. Taft San. Eng. Center, Cincinnati, O. p.76 (1959).

108. Heslop, J.P. and Ray, J.W., J. Insect Physiol., Vol. 3, p.395 (1959).

109. Hess, A. D., and Kenner, G.G., Jr., J. Wildl. Manag., Vol. 11, p.1 (1947).

110. Hickey, J.J., and Anderson, D.W., Science, Vol. 162, p.271 (1968).

111. Hickey, J.J., and Hunt, L. B., J. Wildl. Manag., Vol. 24, p.259 (1960).

112. Hickey, J.J., Keith, J.A., and Coon, F.B., J. Appl. Ecol., Vol. 3 (Supplement on pesticides in the environment and their effects on Wildlife), p.141 (1966).

113. Hoffman, C.H., Biol. Problems in Water Pollution, Tech. Rep. W60-3 Robt. A. Taft San. Eng. Center, Pub. Health Serv., Washington, D.C. (1959).

114. Hoffman, C.H., and Merkel, E.P., J. Econ. Entomol., Vol. 41, p.464 (1948).

115. Hoffman, C.H., and Surber, E.W., Trans. Am. Fish. Soc., Vol. 75, p.48 (1948).

116. Hoffman, C.H., and Surber, E.W., Spec. Sci. Rep. No. 3, Bur. Sport Fish. and Wildl. Dept. of the Interior, Washington, D.C. (1949).

117. Hoffman, C.H. et al., Plant Quar. E-702, Bur. Entomol., Dept. of Agriculture, Washington, D.C. (1946).

118. Hough, W.S., Clancy, D.W., and Pollard, H.N., J. Econ. Entomol., Vol. 38, p.422 (1945).

119. Hoy, W., and Gordon, H.T., J. Econ. Entomol., Vol. 54, p.198 (1961).

120. Hunt, E.G., J. Appl. Ecol., Vol. 3 (Supplement on pesticides in the Environment and their effects on Wildlife), p.113 (1966).

121. Hunt, L.B., J. Wildl. Manag., Vol. 24, p.139 (1960).

122. Hunt, L.B., Circ. 226, Fish and Wildlife Service, Dept. of the Interior, Washington, D. C. (1965).

123. Ide, F.P., Trans. Amer. Fish Soc. Vol. 86, p.208 (1957).

124. Ide, F.P., J. Canad. Fish Research Board, Vol. 24, p.769 (1967).

125. Jackson, W.B., Ecol. Monogr., Vol. 22, p.259 (1952).

126. Jensen, J.A. et al., J. Agr. Food Chem., Vol. 5, p.919 (1957).

127. Johnson, H.E., and Pecor, C., North Am. Wildl. Natur. Resources Conf., Trans., Vol. 34, p.159 (1969).

128. Jones, J.M., and Hayes, J.D., Pl. Pathol., Vol. 16, p.139 (1967).

129. Keener, G.G., and Hess, A.D., J. Wildl. Manag., Vol. 11, p.97 (1947).

130. Keenleyside, M.H.A., J. Fish. Res. Board Can., Vol. 24, p.807 (1967).

131. Keith, J.A., Circ. 226, Fish and Wildlife Service, Dept. of the Interior, Washington, D.C. (1965).

132. Keith J.A., J. Appl. Ecol. Vol. 3 (Supplement on pesticides in the Environment and their effects on Wildlife, p.57 (1966).

133. Kerswill; C.J., and Edwards, H.E., J. Fish. Res. Board Can., Vol. 24, p.709 (1967).

134. King, S.F., Spec. Sci. Rep.: Fisheries No. 399, Fish and Wildlife Service, Dept. of the Interior, Washington, D.C. (1962).

135. Klostermeyer, E.C., and Rasmussen, W.B., J. Econ. Entomol., Vol. 46, p.910 (1953).

136. Kuenen, D.J., Ent. Exp. Appl., Vol. 1, p.147

137. Lackey, J.B., and Steinle, M.L., Pub. Health Rep. No. 7, Dept. of Health, Education, and Welfare, Washington, D.C. (1945).

138. Lauger, P., Martin, H., and Muller, P., Helv. Chim. Acta, Vol. 27, p.892 (1944).

139. Lawrence, H.M., Prog. Fish. Culturist, Vol. 12, p.141 (1950).

140. Lehman, A.J., Assoc. of Food and Drug Officials of the U.S., Quarterly Bull., Vol. 18, p.3 (1954).

141. Lewis, S.E., Nature, Vol. 172, p.1004 (1953).

142. Lewis, S.E., Waller, J.B., and Fowler, K.S., J. Insect Physiol., Vol. 4, p.128 (1960).

143. Lichtenstein, E.P., J. Agr. Food Chem., Vol. 7, p.430 (1959).

144. Linduska, J.P., Audubon Mag., Vol. 54, p.144 (1952).

145. Linduska, J.P., Audubon Mag., Vol. 54, p.248 (1952).

146. Lipke, H., and Kearns, C.W., J. Econ. Entomol., Vol. 53, p.31 (1960).

147. Loosanoff, V.L., Fish. Gas., Vol. 64, p.94 (1947).

148. Lord, F.T., Can Entomol., Vol. 88, p.129 (1956).

149. Macek, K.J., J. Fish. Res. Board Can., Vol. 25, p.1787 (1968).

150. Macek, K.J., J. Fish. Res. Board Can., Vol. 25, p.2443 (1968).

151. Macek, K.J., and McAllister, W.A., Trans. Am. Fish Soc., Vol. 99, p.20 (1970).

152. MacPhee, A.W., Chisholm, D., and MacEachern, C.R., Canad. J. Soil Sci., Vol. 40, p.59 (1960).

153. Matsumura, F., and O'Brien, R.D., J. Agr. Food Chem., Vol. 14, p.36 (1966).

154. Menhinick, E.F., Ecology, Vol. 43, p.556 (1962).

155. Menzel, D.B. et al., J. Econ. Entomol., Vol. 54, p.9 (1961).

156. Middleton, F.M., and Rosen, A.A., Pub. Health Rep., Vol. 71 p.1124 (1956).

157. Middleton, F.M., Proc. Conf. Physiol. Aspects of Water Quality Pub. Health Serv., Washington, D.C. (1960).

158. Miskus, R.P., Blair, D.P., and Casida, J.E., J. Agr. Food Chem., Vol. 13, p.481 (1965).

159. Mullins, L.J., Science, Vol. 122, p.118 (1965).

160. Muncy, R.J., and Oliver, A.D., Trans. Am. Fish Soc., Vol. 92, p.428 (1963).

161. Munson, S.C., and Yeager, J.F., J. Econ. Entomol., Vol. 38, p.618 (1945).

162. Narahashi, T., and Yamasaki, T., J. Physiol. (London), Vol. 151, p.75 (1960).

163. Narahashi, T., and Yamasaki, T., J. Physiol. (London), Vol. 152, p.122 (1961).

164. Nash, R.G., and Woolson, E.A., Science, Vol. 157, p.924 (1967).

165. Neave, D.J., and Wright, B.S., J. Wildl. Manag., Vol. 33, p.1015 (1969).

166. Nelson, A.L., and Surber, E.W., Spec. Sci. Rep. No. 41, Bur. Sport Fish and Wildl., Dept. of the Interior, Washington, D.C. (1947).

167. Newcomer, E.J., and Dean, E.P., J. Econ. Entomol., Vol. 39, p.783 (1946).

168. O'Brien, R.D. and Dannelley, C.E., J. Agr. Food Chem., Vol. 13, p.245 (1965).

169. O'Brien, R.D., and Matsumura, F., Science, Vol. 146, p.657 (1964).

170. Odum, W.E., Woodwell, G.M., and Wurster, C.F., Science, Vol. 164, p.576 (1969).

171. Ogilvie, D.M., and Anderson, J.M., J. Fish. Res. Board Can., Vol. 22, p.503 (1965).

172. Ortega, P. et al., Public Health Monograph No. 43, Pub. Health Service, Washington, D.C. (1956).

173. Ortelee, M.F., Jour. A.W.W.A., Vol. 51, p. 984 (1959).

174. Ozburn, G.W., and Morrison, F.O., Can J. Zool., Vol. 42, p.519 (1964).

175. Pagan, C. and Hageman, R.H., Science, Vol. 112, p.222 (1950).

176. Parker, V.H., Biochem. J., Vol. 77, p.74 (1960).

177. Pauling, L., and Pressman, D., J. Am. Chem. Soc., Vol. 67, p.1003 (1945).

178. Perry, A.S., Miller, S., and Buckner, A.J., J. Agr. Food Chem., Vol. 11, p.457 (1963).

179. Peterson, J.E., and Robison, W.H., Toxicol. Appl. Pharmacol., Vol. 6, p.321 (1964).

180. Pielou, D.P., Can J. Zool., Vol 38, p.1147 (1960).

181. Pimental, D., J. Econ. Entomol., Vol. 54, p.108 (1961).

182. Porter, R.D., and Wiemeyer, S.N., Science, Vol. 165, p.199 (1969).

183. Princi, F., Bull, Hygiene, Vol. 30, p.527 (1955).

184. Robbins, C.S., Springer, P.F., and Webster, C.G., J. Wildl. Manag., Vol. 15, p.213 (1951).

185. Roeder, K.D., and Weiant,E.A., Science, Vol. 103, p.304 (1946).

186. Rogers, E.F. et al., J. Am. Chem. Soc., Vol. 75, p.2991 (1953).

187. Rudd, R.L., and Genelly, R.E., Game Bull. No. 7, Calif. Dept. Fish and Game, Sacramento, Calif. (1956).

188. Salkeld, E.H., Nature, Vol. 166, p.608 (1950).

189. Sanders, H.O., Tech. Paper 25, Bur. Sport Fish and Wildl., Dept. of the Interior, Washington, D.C. (1969).

190. Sawyer, C.N., Sewage and Industrial Wastes, Vol. 30, p.757 (1958).

191. Smith, S.I., Weber, C.W., and Reid, B.L., Poultry Sci., Vol. 48, p.1000 (1969).

192. Steiner, A. Arnold, C.H., Summerland, S.A., J. Econ. Entomol., Vol. 37, p.156 (1944).

193. Sternburg, J., and Kearns, C.W., Science, Vol. 116, p.144 (1952).

194. Sternburg,J., and Hewitt, P., J. Insect Physiol., Vol. 8, p.643 (1962).

195. Sternburg, J., Ann. Rev. Entomol., Vol. 8, p.19 (1963).

196. Stickel, L.F., J. Wildl. Manag., Vol. 10, p.216 (1946).

197. Stickel, L.F., J. Wildl. Manag., Vol. 15, p.161 (1951).

198. Stickel, L.F., Stickel, W.H., and Christensen, R. Science, Vol. 151, p.1549 (1966).

199. Sacklin, J.A., Terriere, L.C., and Remmert, L.F., Science, Vol. 122, p.378 (1955).

200. Surber, E.W., Prog. Fish Cult., Vol. 9, p.154 (1947).

201. Surber, E.W., Prog. Fish Cult., Vol. 10, p.125 (1948).

202. Surber, E.W., Sixth Ann. Poll. Abatement Conf., Mfg. Chem. Assoc., Inc., Washington, D.C. (1951).

203. Surber, E.W., and Friddle, D.D., Trans. Am. Fish. Soc., Vol. 76, p.315 (1946).

204. Surber, E.W., and Hoffman, C.H., Spec. Sci. Rep. No. 4, Bur. Sport Fish and Wildl., Dept. of the Interior, Washington, D.C. (1949).

205. Tarpley, W.A., J. Econ. Entomol., Vol. 51, p.780 (1958).

206. Tarzwell, C.M., J. National Malaria Soc., Vol. 7, p.199 (1948).

207. Tarzwell, C.M., Pub. Health Reports, Vol. 65, p.231 (1950).

208. Tombes, A.S., and Forgash, A.J., J. Insect Physiol., Vol. 7, p.216 (1961).

209. Vance, B.D., and Drummond, W., J. Am. Water Works Assoc., Vol. 61, p.360 (1969).

210. Vinson, S.B., Boyd, C.E., and Ferguson, D.E., Science, Vol. 139, p.217 (1963).

211. Wadsworth, J.R., Vet. Med., Vol. 48, p.412 (1952).

212. Wallace, G.J., Etter, A.G., and Osborne, D.R., Mass. Audubon, Vol. 48, p.116 (1964).

213. Waller, G.D., J. Econ. Entomol., Vol. 62, p.189 (1969).

214. Waller, J.B., and Lewis, S.E., J. Insect Physiol., Vol. 7, p.315 (1961).

215. Warner, K., "Summary of Effects of DDT Spraying on Northern Maine Trout Streams, 1960." Maine Dept. of Inland Fisheries and Game, Augusta, Maine (1960).

216. Warner, K., and Fenderson, O.C., "Effects of Forest Insect Spraying on Northern Maine Trout Streams." Maine Dept. of Inland Fisheries and Game, Augusta, Maine (1960).

217. Washburn, G.N., Mich. Acad. Sci., Vol. 33, p.181 (1947).

218. Way, M.J., Bull. Entomol. Res., Vol. 40, p.279 (1949).

219. Webbe, G., and Shute, G.T., Am. Trop. Med. and Parasitol., Vol. 53, p.47 (1959).

220. Wheeler, E.H., and LaPlante, A.A., J. Econ. Entomol., Vol. 39, p.211 (1946).

221. Wiemeyer, S.N., and Porter, R.D., Nature, Vol. 227, p.737 (1970).

222. Wisecup, C.B. et al., J. Econ Entomol., Vol. 39, p.52 (1946).

223. Woglum, R.S. et al., J. Econ. Entomol., Vol. 40, p.818 (1947).

224. Woodwell, G.M., Wurster, C.F., and Issacson, P.A., Science, Vol. 156, p.821 (1967).

225. Wright, B.S., J. Wildl. Manag., Vol. 29, p.172 (1965).

226. Wurster, C.F., Jr., Wurster, D.H., and Strickland, W.N., Science, Vol. 148, p.90 (1965).

227. Wurster, C.F., Jr., Science, Vol. 159, p.1474 (1968).

228. Yothers, M.A., and Carlson, F.W., J. Econ. Entomol., Vol. 41, p.515 (1948).